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PREFACE

Volume 132 contains five excellent reviews that have broad international relevance related to soil and water quality, environmental sustainability, food production, and climate change. Chapter 1 deals with the important role that wetlands and their restoration play in water quality on the Chesapeake Bay coastal plain. Chapter 2 deals with the impact of nitrogenous gas emissions from soils on greenhouse gases in the environment. Chapter 3 covers the important topic of arsenic contamination of groundwater in Eastern Asia, with emphasis on hydrological aspects. Chapter 4 discusses the use of soil spectroscopy as an alternative to wet chemistry for soil monitoring. Chapter 5 discusses the occurrence, detection, and molecular and metabolic characterization of heat-resistant fungi in soils and plants and their risk to human health.

I appreciate the fine contributions of the authors.

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CHAPTER ONE

Wetland Restoration and Creation for Nitrogen Removal: Challenges to Developing a Watershed-Scale Approach in the Chesapeake Bay Coastal Plain

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Abstract

Concern for the health of the Chesapeake Bay and the establishment of the Bay Total Maximum Daily Load have led to growing interest in restoring and creating wetlands to mitigate agricultural nitrogen inputs. All Bay states have included wetland restoration in their watershed implementation plans (WIPs) to help meet their required reduction in nitrogen loading. In agricultural areas of the coastal plain, efforts to develop a watershed-scale approach to siting and designing wetlands have been met with considerable

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challenges. Nitrate loss is primarily attributed to base-flow conditions, and groundwater flow is multidimensional and highly variable, so accounting for nitrate transport connectivity between agricultural N source areas and potential wetland restoration areas is difficult. Socioeconomic and political challenges also constrain implementation. Our ability to account for subsurface connectivity may be improved with better assessment of hydrologic connectivity in areas with artificial drainage, catchment-scale studies of hydrogeomorphic predictions of hydrologic connectivity, and improved use of geospatial data. A coordinated monitoring program would improve our ability to estimate wetland nitrogen removal efficiencies across environmental and management conditions. The addition of a requirement that water quality should be an explicit objective of restorations included within WIP accounting would avoid the inclusion of projects with minimal water quality benefits. Research is also needed on farmer attitudes in the Chesapeake Bay watershed toward wetlands for water quality protection. These proposed actions would improve our ability to understand and implement wetland restoration as a component of our response to meet water quality objectives.

1. INTRODUCTION

Nitrogen losses due to increases in agricultural applications of fertilizer and manure over the past 60 years have contributed to eutrophication, hypoxia, and habitat loss in the Chesapeake Bay. The Chesapeake Bay receives an estimated 1.32×10^8 kg N per year, with agriculture contributing more than half of this load (Ator et al., 2011). In 2010, the US Environmental Protection Agency established the Chesapeake Bay Total Maximum Daily Load (TMDL), a "pollution diet" for the Chesapeake Bay and the region's creeks, streams, and rivers. The TMDL sets pollution limits on nitrogen, phosphorus, and sediment necessary to meet water quality standards in the Bay and its tidal rivers. For nitrogen, this limit is set at 84.3 million kg N per year. Measures to achieve the TMDL must be in place by 2025 with 60% completion by 2017 (US Environmental Protection Agency, 2010). Bay states are required to develop watershed implementation plans (WIPs) that document how local jurisdictions will work with state and federal governments to control nutrient and sediment loads and meet TMDLs. WIPs are to be submitted to the EPA in three phases with increasing level of detail: Phase I implementation plans in November 2010, Phase II in March 2012, and Phase III in 2017.

Reducing nutrient loading from agricultural sources will require a broad suite of practices, including on-farm, edge-of-field, and off-site practices. One potential edge-of-field or off-site strategy is targeted restoration and creation of wetlands. Wetlands can function as removal sites or "sinks" for

N primarily by promoting denitrification, a microbial process by which nitrate is converted to gaseous nitrogen products thereby permanently removing N from the soil-water environment. The 2014 Chesapeake Watershed Agreement includes the outcome of creating or reestablishing 34,400 hectares of tidal and nontidal wetlands and enhancing the function of an additional 60,700 hectares of degraded wetlands by 2025 (Chesapeake Bay Program, 2014).

The restoration and creation of wetlands for controlling nonpoint source pollution in agricultural watersheds has been widely investigated (Phipps and Crumpton, 1994; Kovacic et al., 2000; Jordan et al., 2003; Vellidis et al., 2003; Hernandez and Mitsch, 2007; Poe et al., 2003). For over 20 years, research in this area has emphasized the need for a watershed-scale approach to siting and designing wetlands in order to optimize performance and meet water quality goals (Van der Valk and Jolly, 1992; Woltemade, 2000; Crumpton, 2001; Mitsch et al., 2001; Zedler, 2003; De Steven and Lowrance, 2011; Osmond et al., 2012; Passeport et al., 2013). In addition to biological and physical constraints, wetland restoration planning requires consideration of political, economic, and social factors that may pose barriers to implementation and are best addressed at the watershed scale. Wetland restoration must also be considered within the context of the broader response strategy, as one of many potential ways to address agricultural nonpoint source pollution. Van der Valk and Jolly (1992) outlined a set of recommendations for research on the use of wetlands to address nonpoint source pollution:

- Whole watershed demonstration studies
- Studies of effectiveness of restored/created wetlands
- Landscape simulation models of origin/movement of nonpoint source pollution
- Studies on site selection and design criteria
- Studies of farmers' and local business/community leaders' attitudes toward landscape approach
- Studies exploring legal and public policy issues of wetland restoration programs
- Studies evaluating the costs and benefits of this approach

There has been little advancement in these research areas in the Chesapeake Bay watershed to date. Wetland projects are still planned primarily at the scale of the individual property. However, in recent years, environmental groups have initiated pilot projects in parts of the watershed to demonstrate the application of a watershed approach to wetland and stream

restoration, with applications in both conservation planning and compensatory mitigation (Wilkinson et al., 2013; The Nature Conservancy, 2013).

This paper addresses some of the barriers to implementing wetland restoration/creation practices in the region, discusses challenges to developing a watershed approach for treating nitrate, and recommends ways to overcome these challenges. We focus on the coastal plain portion of the Chesapeake Bay watershed because of the great potential for wetland restoration in this region due to the widespread agricultural land use and history of artificial drainage of wetlands.

2. BIOLOGICAL AND PHYSICAL CHALLENGES

In taking a watershed-scale approach to siting and designing wetlands for mitigating nitrate runoff, we should consider where on the landscape agricultural nitrate is being delivered to streams and where the topography and soils are most suitable for wetland establishment. Where these factors coincide, there is an opportunity to restore or create wetlands that will be effective at removing nitrate and improving water quality. We have identified two major biological and physical challenges for the siting of wetlands for nitrate removal: (1) accounting for subsurface connectivity between nitrogen sources and wetlands and (2) estimating how effective wetlands will be at removing nitrate in order to demonstrate the benefits of targeted wetland restoration and compare alternative watershed plans.

2.1 Subsurface Connectivity between Nitrogen Sources and Wetlands

A wetland can only be effective at mitigating N if there is hydrologic connectivity between the N source and the wetland site. Nitrogen transport in the coastal plain of the Chesapeake Bay watershed is often subsurface and varies in depth and transport time. Also, the direction of flow does not necessarily follow topographic patterns. Subsurface hydrologic connectivity is not consistent over time and may be altered through hydrologic restoration.

The Delmarva Peninsula forms the largest portion of the Mid-Atlantic Coastal Plain portion of the Chesapeake Bay watershed. The flat topography and permeable soils of much of the Delmarva Peninsula favor subsurface flow (Staver and Brinsfield, 1998; Hamilton et al., 1993). Nitrate leaching from the crop rooting zone during winter months contributes to elevated groundwater nitrate concentrations (Staver and Brinsfield, 1998). The peninsula is underlain by a wedge of unconsolidated sediments comprised

of a surficial unconfined aquifer ranging from less than 6 m to greater than 30 m thick (Hamilton et al., 1993) underlain by a series of confined aquifers (Cushing et al., 1973) (Figure 1). Due to the high permeability of aquifer sediments, groundwater is well oxygenated throughout most of the aquifer

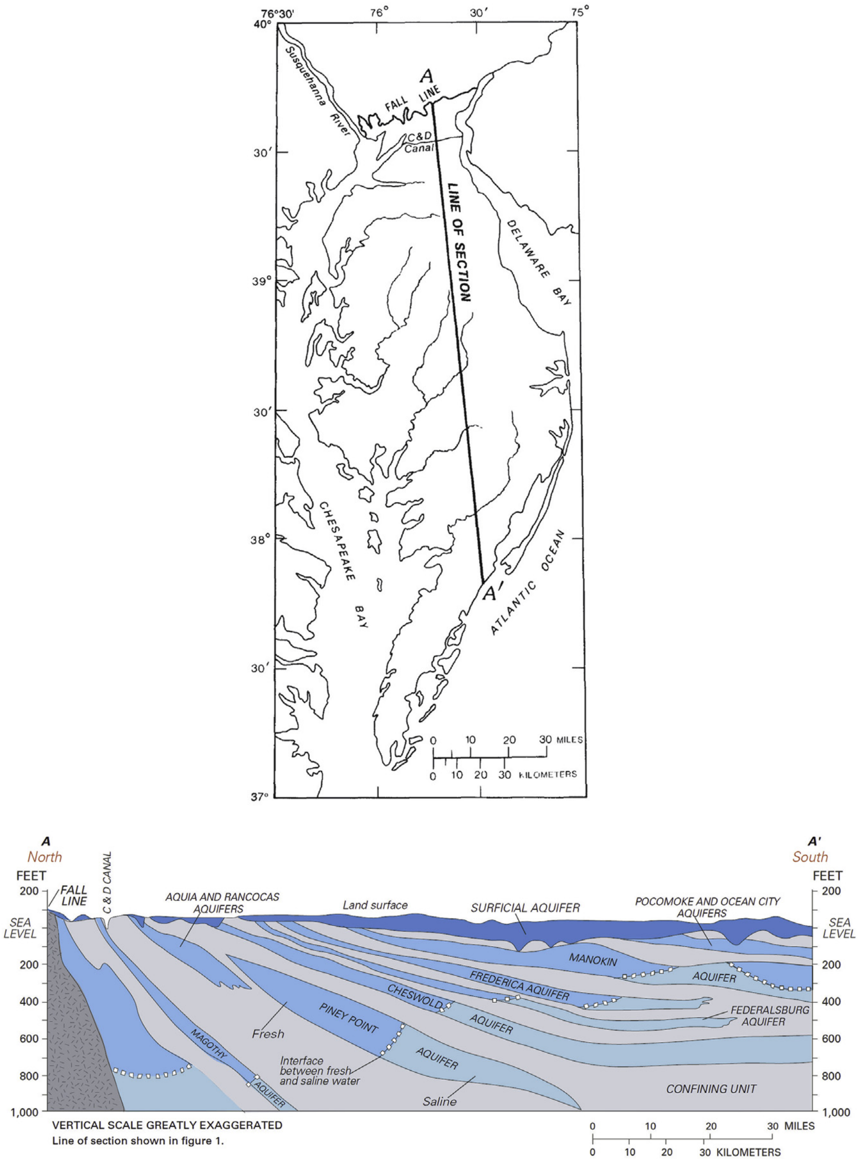

Figure 1 Stratigraphies of the Delmarva Peninsula. An extensive surficial aquifer overlies a series of confined aquifers. *Image produced by the US Geological Survey, Hamilton et al. (1991) with modification by Denver et al. (2004).*

and elevated nitrate concentrations are found even near the base of the surficial aquifer (Hamilton et al., 1993; Debrewer et al., 2007). Seventy percent of the nitrogen flux in headwater streams is attributable to base-flow nitrate flux (Ator and Denver, 2012). Debrewer et al. (2007) reported that median nitrate concentrations in groundwater are greater than 5 mg L^{-1} and often higher than 10 mg L^{-1} in wells placed at a median depth of 6 m below the surface in agricultural areas. Similar nitrate concentrations in deeper groundwater (14 m below surface) reflect recharge in upgradient agricultural land (Hamilton et al., 1993; Debrewer et al., 2007). Mitigating nitrate loss is further complicated by the long travel time required for deep groundwater to move through the surficial aquifer; nitrate may remain in groundwater for decades to centuries before discharging into streams (Bohlke and Denver, 1995).

Depressional wetlands have been the focus of much of the wetland restoration efforts on the Delmarva Peninsula. Two common wetland restoration techniques are commonly used in these landforms: (1) plugging agricultural drainage ditches and (2) excavating the topsoil and building a berm ("scraping"). In some cases, ditch-plugging may not be effective for groundwater nitrate mitigation. When a ditch is plugged, the water level in the ditch rises reducing the hydraulic gradient between the ditch and the groundwater. Groundwater that previously flowed into the ditch may begin to flow in another direction, potentially bypassing the wetland treatment area (T. Jordan, 2013, pers. comm.). Scraping is a more common practice in Maryland, but it is also unclear how this method affects N transport and processing. By compacting the subsoil during excavation, scraping likely affects soil properties such as bulk density and pore size distribution, with important implications for the fate of subsurface N.

Potential restoration sites are evaluated through site visits and examination of topographic and soils maps, but these methods often do not adequately account for subsurface hydrologic flow paths, which are a major transport pathway of nitrate in coastal plain regions of the Chesapeake Bay watershed (Hamilton et al., 1993; Staver and Brinsfield, 1996; Sanford et al., 2012).

Tile and ditch drainage are common in poorly drained agricultural areas, where wetlands are most likely to be successfully established. Artificial drainage networks can provide a conduit for the rapid and continuous delivery of nitrate to surface waters. Few studies have been published on the effects of artificial drainage on water quality on the Mid-Atlantic Coastal Plain (Schmidt et al., 2007; Vadas et al., 2007; Kleinman et al., 2007;

Needelman et al., 2007), but recent research in Maryland has shown that ditch depth and the presence of subsoil clay-rich horizons can affect transport of nitrate through ditches (Vadas et al., 2007; Schmidt et al., 2007). Shallow ditches (∼0.5 m) function mainly as conduits for surface water during runoff-generating rainfall events, receiving few subsurface inputs (Schmidt et al., 2007). Deeper ditches (∼1 m) drain proportionately more water due to continuous subsurface flow inputs, and nitrate loss increases linearly with drainage outflow (Schmidt et al., 2007). The presence of low conductivity clay-rich horizons can cause water tables to perch temporarily following rain events, promoting rapid, lateral movement of water to ditches (Vadas et al., 2007). Old drainage ditches within restored wetlands can also have important implications for nitrate transport. Vellidis et al. (2003) identified preferential flow paths associated with old drainage ditches that permitted groundwater nitrate plumes to flow deep within wetland soils, limiting interaction with the biologically active rooting zone.

Nitrate-enriched groundwater from agricultural fields is generally expected to flow through riparian areas to streams laterally through the shallow subsurface (Lowrance et al., 1997; Gold et al., 2001) (Figure 2). However, subsurface flow may be more heterogeneous and asymmetrical than this general model predicts (Angier et al., 2005; Gold et al., 2001). In some locations, deeper groundwater or preferential flow paths can deliver nitrate directly to streams with limited opportunity for N processing (Gold et al., 2001; Angier et al., 2005) (Figure 2). Thus, in areas where subsurface flow is the primary transport of nitrate, the effectiveness of wetland best management practices (BMPs) to mitigate nitrate depends on our ability to understand how nitrate is moving in the subsurface.

Subsurface flow may follow preferential flow pathways controlled by variations in soil and aquifer characteristics horizontally and with depth. Both macropore flow and funneled flow have important implications for solute transport. Kung (1990) identified preferential flow triggered by funnels created by abrupt textural discontinuities and inclined bedding planes as the dominant mechanism in a sandy vadose zone in Wisconsin. Funneled flow allows for rapid transport of contaminants and can be difficult to detect using common solute sampling techniques (Kung, 1990).

In a first-order riparian zone in an agricultural catchment in the Mid-Atlantic Coastal Plain, Angier et al. (2005) found that much of the groundwater nitrate was delivered to a stream through zones of concentrated flow. The authors observed higher hydraulic conductivities associated with 5-cm thick sand layers 80 and 120 cm below the surface within otherwise low

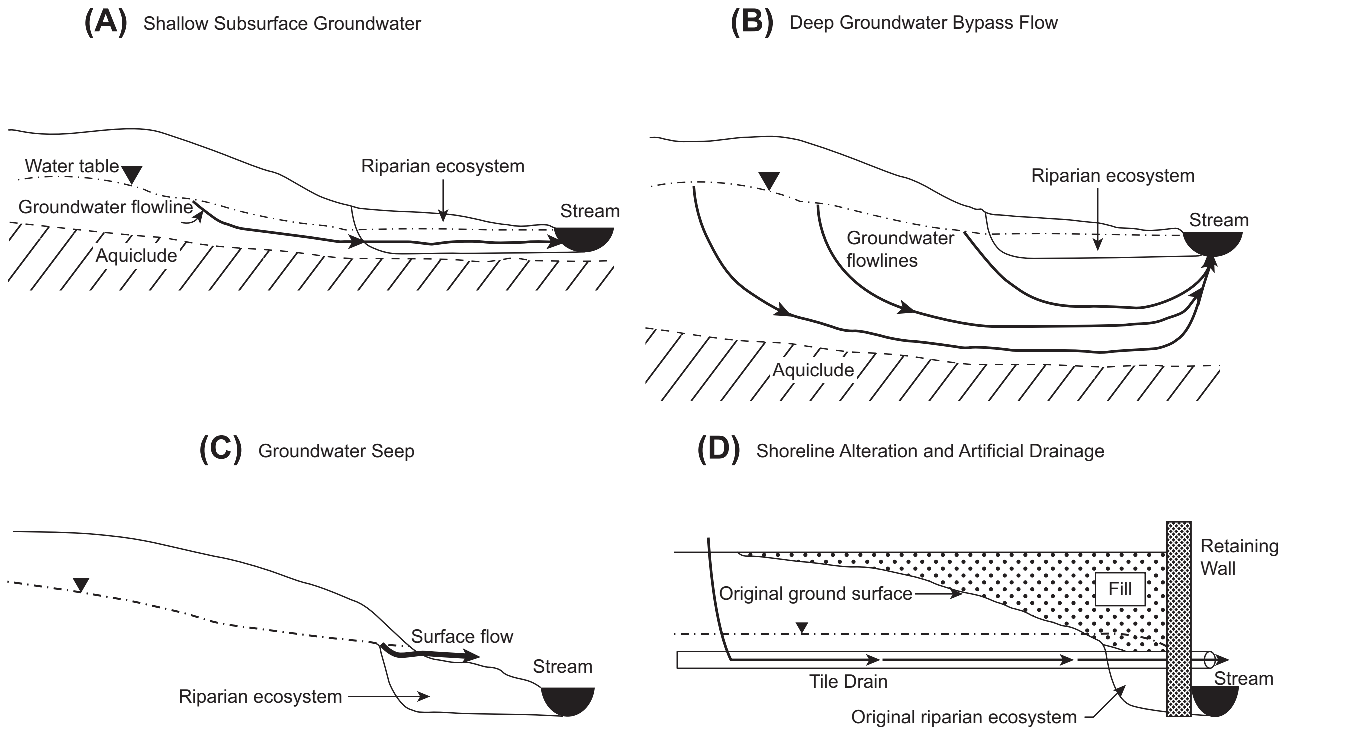

Figure 2 Groundwater flow paths through riparian areas can control the delivery of nitrate-enriched groundwater to streams. (A) Substantial interaction of ground water with biologically active zone in shallow aquifers; (B) Direct upwelling to streams in deep aquifers; (C) Bypass flow due to surface seeps; (D) Bypass flow due to filling and artificial drainage. *Reprinted with permission, Gold et al. (2001).*

conductivity fine-textured wetland soils. These layers probably acted as preferential transport sites, delivering groundwater to discharging macropores along the stream (Angier et al., 2005). Upwelling zones supplied a disproportionate amount of total stream flow, including a single upwelling area that comprised 0.006% of the riparian area but generated on average 4% of total stream flow (Angier et al., 2005). This example illustrates the importance of being able to identify surface features and soil properties that control hydrologic connectivity. Traditional models of horizontal matrix flow were inadequate for describing the connectivity of this riparian ecosystem where significant amounts of nitrate reached the stream channel.

Differences in soil and aquifer hydraulic properties and the depth of groundwater flow have important implications for siting and designing wetlands. Where the surficial aquifer is thick, nitrate-rich groundwater may flow below the wetland treatment area, limiting N removal potential (Bohlke and Denver, 1995; Gold et al., 2001). Alternatively, groundwater may pass through reducing sediments at depth where nitrate removal by denitrification may occur before discharging into streams (Bohlke and Denver, 1995). Even adjacent watersheds with similar groundwater nitrate levels can display significant differences in groundwater flow patterns due to variation in local aquifer characteristics (Bohlke and Denver, 1995).

Depressional wetlands are common throughout the upper and middle portions of the Delmarva Peninsula (Clearwater et al., 2000; Fenstermacher et al., 2014) in counties dominated by agricultural land use. The complexity of N fate and transport complicates evaluating the effects of depressional wetlands on downstream water quality (Denver et al., 2014). In flat landscapes, groundwater flow paths do not always follow topographic gradients; seasonal reversals in the direction of groundwater flow can cause shallow groundwater to move away from the wetland to the agricultural upland (Denver et al., 2014) (Figure 3). Due to the multidimensionality of groundwater flow and variability in reducing conditions, limited geochemical and hydrologic measurements along a presumed hydrologic transect are often insufficient for determining the potential for nitrate interception and removal in wetlands (Denver et al., 2014).

Soil characteristics and geomorphology can provide insight into how aquifer attributes affect groundwater flow and nitrate flux (Gold et al., 2001). For example, research on riparian zones has demonstrated that organic/alluvial deposits show a greater capacity for groundwater nitrate removal than till deposits (Rosenblatt et al., 2001; Gold et al., 2001). In a study of riparian zones in different hydrogeologic settings, Vidon and Hill

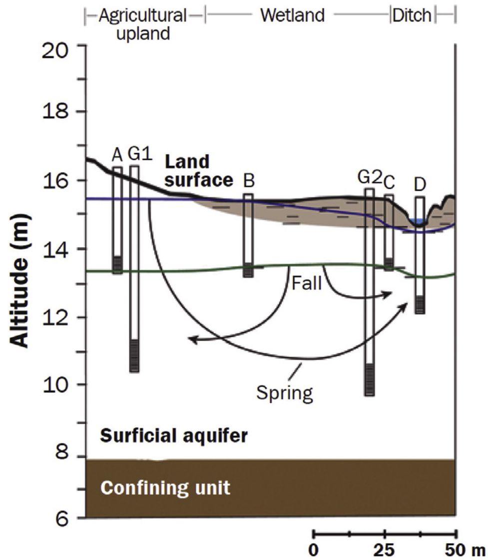

Figure 3 Cross section of a prior-converted cropland site illustrating seasonal reversal in hydrologic gradient. *Reprinted, Denver et al. (2014).*

(2004) demonstrated how landscape characteristics, including upland aquifer depth, slope, and riparian soil texture, can affect the magnitude and duration of nitrate inputs and the potential for nitrate removal in riparian zones. This study highlighted the importance of hydrologic connectivity between upland and riparian areas for nitrate removal; riparian sites with gentle topography, confining layers, and potentially high nitrate removal rates were not important nitrate sinks because of limited water and nitrate inputs from uplands. Method of restoration can affect soil characteristics and the potential for nitrate removal in wetlands as well. For example, confining layers created by the addition of clay or compaction of soils during restoration may limit interaction between anoxic wetland sediments and nitrate in groundwater (Denver et al., 2014).

There is great interest in using remote sensing and geospatial technology to target and monitor wetland restorations using information on topography, hydrology, land cover/use, and soil and aquifer properties. These technologies have successfully been used to predict surface hydrologic processes (Lang et al., 2012, 2013), but developing predictions of groundwater connectivity based on landscape and soil characteristics is more challenging. High resolution Light Detection and Ranging (LiDAR) data allow us to identify terrain attributes with high vertical (15–100 cm) and horizontal

accuracy (50–200 cm), and can significantly improve detection of surface hydrologic connections (Lang et al., 2012). Soil data from the Soil Survey Geographic Database (SSURGO) contain information on soil hydrologic properties to a depth of approximately 2 m including hydric rating, soil texture, hydraulic conductivity, available water capacity, hydrologic group, drainage class, organic matter, and bulk density. SSURGO data, however, are considerably coarser (1:12,000 to 1:65,360) than LiDAR data, and their applications in land management planning are limited by the spatial aggregation of soil components. Differences in resolution can lead to problems when overlaying GIS data for mapping and spatial analysis. Spatial aggregation of SSURGO components also creates problems when mapping soil properties for use in landscape analysis. For example, when hydric soil rating is overlain on top of LiDAR data, the coarse resolution of the soil data can conceal the subtle variations in topographic indices of wetness depicted by the LiDAR data. Furthermore, map units often include major and minor components with both hydric and nonhydric soils. A common summarization technique is to assign hydric rating categories based on the cumulative percent composition of all components of a map unit rated as hydric (Figure 4). However, such summarization does not address issues of scale. Due to spatial heterogeneity in hydrologic flow paths and geochemical conditions, finer scale soil property maps would help natural resource managers characterize near-surface hydrologic connectivity between agricultural uplands and wetlands and predict where geochemical conditions may be optimal for restoring wetlands to capture and remove nitrate. The challenge

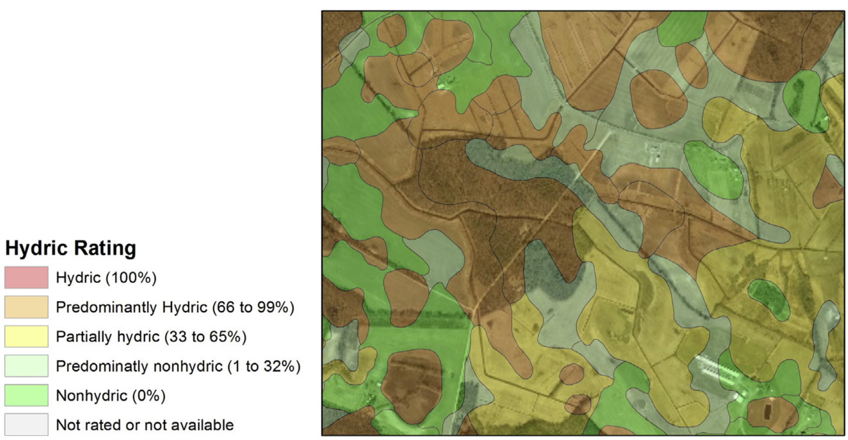

Figure 4 NRCS Web Soil Survey hydric soil rating (Soil Survey Staff, 2014b).

on the Mid-Atlantic Coastal Plain, however, remains that groundwater carrying abundant nitrate is often considerably deeper than 2 m, and aquifer characteristics have not been mapped to sufficient resolution or consistency to predict flow patterns (Ator et al., 2012). Using information compiled from geophysical and lithologic logs taken across the Delmarva, geologists have mapped the base of the surficial aquifer at a resolution of 762 m^2, which is too coarse for use at local scales (Andreason et al., 2013).

2.1.1 Proposed Approach

Although we do not currently have the tools to accurately predict groundwater connectivity between N sources and wetlands at a watershed-scale, there are several actions we can take to better account for subsurface N transport when siting and designing BMPs. The following research is recommended in Mid-Atlantic Coastal Plain watersheds to enhance the implementation of appropriate N management strategies (detailed below):

1. Assessing hydrologic connectivity in areas with artificial drainage
2. Catchment-scale studies of hydrogeomorphic predictions of hydrologic connectivity
3. Improved use of geospatial data for predicting subsurface connectivity between N sources and wetlands

2.1.1.1 Assessing Hydrologic Connectivity in Areas with Artificial Drainage

Hydrologic connectivity in the Mid-Atlantic Coastal Plain is highly influenced by artificial drainage, but there have been a limited number of studies examining nitrate delivery to and from artificial drainage ditches (Schmidt et al., 2007; Vadas et al., 2007). Schmidt et al. (2007) found that shallow ditches on a research farm in southern Delmarva received negligible amounts of subsurface flow inputs; therefore management practices designed to impact groundwater flow would be ineffective in these locations. By contrast, wetlands, riparian buffers, denitrification walls, and controlled drainage structures would likely be effective at mitigating groundwater nitrate moving into deep ditches (Schmidt et al., 2007). Further research examining factors affecting hydrologic transport of N to ditches, including the relative importance of lateral matrix flow and preferential flow in different hydrogeologic settings, would help us identify opportunities to capture and treat nitrate before it reaches the ditch. In cases where ditches intercept groundwater nitrate, wetland restoration adjacent to ditches may help maintain anoxic conditions beneath ditches (Denver et al., 2014), thereby encouraging denitrification in ditch soils and sediments. Controlled

drainage structures installed in ditches manage drainage outflow and may enhance denitrification. We recommend testing different designs in order to maximize the effectiveness of these BMPs.

2.1.1.2 Catchment-Scale Studies of Hydrogeomorphic Predictions of Hydrologic Connectivity

Different hydrogeomorphic regions on the Mid-Atlantic Coastal Plain display unique groundwater flow and water quality patterns (Hamilton et al., 1993). The highest nitrate concentrations are found in groundwater beneath agricultural areas where the soils and surficial aquifer are composed of sandy, permeable sediments with little clay. Groundwater nitrate is particularly high in the "well-drained uplands," a hydrogeomorphic region characterized by narrow incised streams, deep water tables (>3 m below land surface), and oxic groundwaters. In the "poorly drained uplands" where stream incision is minimal and the water table is within 3 m of land surface, mixing of aerobic and anaerobic groundwater results in lower nitrate concentrations. The lowest groundwater nitrate concentrations are found in regions where organic matter is abundant and clay and silt deposits inhibit downward flow, including the "fine-grained lowlands" and parts of the "surficial confined" region (Hamilton et al., 1993). Different N management strategies may be targeted in different hydrogeomorphic regions. Opportunities for wetland restoration BMPs will likely be greatest in the "poorly drained uplands" and "surficial confined" regions, where the water table is close to the surface and much of the land is in agriculture. This includes much of the central peninsula (Figure 5).

Where sediments are highly permeable and nitrate contamination in deeper groundwater is high, identification of groundwater discharge sites may be important for capturing nitrate. Where clay and silt deposits inhibit downward flow, identification of preferential flow paths in the shallow subsurface may be more important for intercepting nitrate, and it may be more feasible to predict subsurface connectivity based on local variability in soil and landscape attributes. Soil moisture predictions are significantly improved when both soil morphological properties and terrain attributes are considered (Takagi and Lin, 2012). The relative importance of soil and terrain parameters in controlling soil moisture varies seasonally and with depth (Takagi and Lin, 2012). Therefore, catchment-scale studies of nitrate transport in different landscape settings are needed to improve our understanding of how topography and soil characteristics affect seasonality of hydrologic connections and the direction and depth of subsurface flow paths.

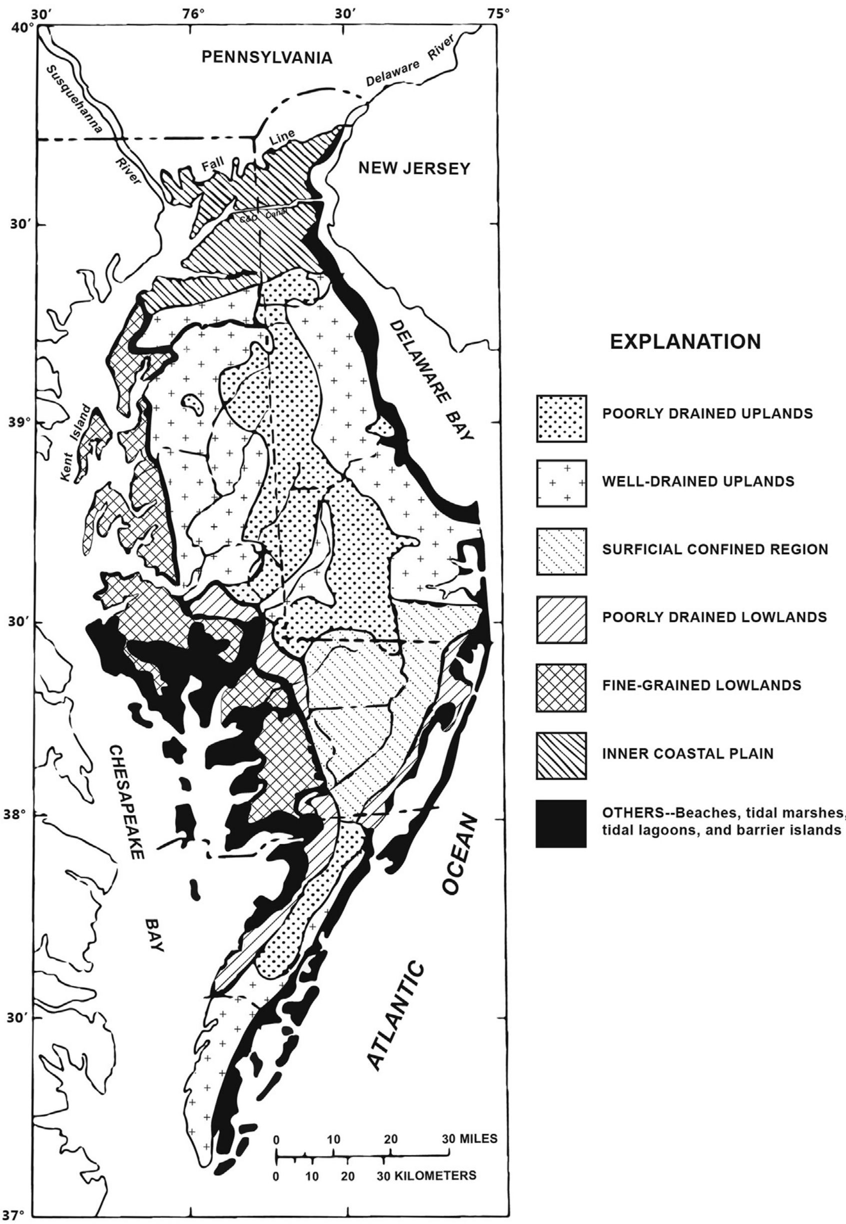

Figure 5 Hydrogeomorphic regions in the surficial aquifer in the Delmarva Peninsula. *Image produced by the US Geological Survey, modified from Hamilton et al. (1993).*

Assumptions of lateral subsurface flow need to be reexamined and better accounting of vertical flow processes and mixing of deeper "old" and shallower "new" groundwater is needed to improve our understanding of subsurface nitrate loss to surface waters. Trends in water chemistry can reveal patterns in nitrate fate and transport. McCarty et al. (2014) found that the relationship between nitrate-N concentration and metolachor metabolite, a stable, water-soluble herbicide degradation product, could be used to distinguish between dilution and denitrification effects on nitrate concentrations in surface waters of the Choptank River watershed. These patterns can help us identify watersheds where denitrification may be enhanced by restoring wetlands (McCarty et al., 2014).

In addition to nitrate removal, wetlands may improve water quality in local streams through mixing and dilution (Denver et al., 2014). Forested wetlands upgradient from agriculture can be a source of low nitrate water to downstream waters, diluting nitrate concentrations and improving regional water quality (Denver et al., 2014). Thus, identifying opportunities to dilute groundwater nitrate by restoring hydrology to ditched forested wetlands will likely be an important component of a watershed approach to wetland restoration.

2.1.1.3 Improved Use of Geospatial Data for Predicting Subsurface Connectivity between N Sources and Wetlands

The success of a watershed approach to wetland restoration and creation will depend greatly on the effective use of geospatial data. Recent advances in geospatial technology and greater access to high resolution data allow for better mapping of landscape factors influencing the fate and transport of nitrate. New GIS-based targeting tools are continually being developed to identify priority areas for wetland restoration (White and Fennessy, 2005; Tomer et al., 2013; Hunter et al., 2012). There are several ways to improve current use of geospatial data to better identify areas where the hydrology may favor N removal by restored/created wetlands. These are explained in the following sections.

2.1.1.3.1 Expanded Use of LiDAR Data and Topographic Indices Derived from LiDAR High-resolution LiDAR data are available for much of the Mid-Atlantic Coastal Plain and can improve detection of saturated areas in the landscape and hydrologic connections between wetlands and surface waters (Lang et al., 2012). Tomer et al. (2013) used LiDAR topographic data in combination with output from water quality models

to identify feasible locations for wetlands in an Illinois watershed and estimate watershed nitrate loads if they are constructed. Topographic metrics derived from LiDAR can be used to predict spatial patterns in soil saturation and map wetlands (Lang et al., 2013). The topographic wetness index, which is based on upslope contributing area and slope, can be generated using a number of different flow routing algorithms. In a raster layer, there are eight directions in which water can flow. GIS programs often use the D8 algorithm, which directs water to the steepest downslope neighboring cell. Other algorithms use more complex decision rules to direct flow to neighboring cells. Distributed flow patterns may better predict flow in relatively flat landscapes (Lang et al., 2013). The topographic wetness index will be most useful in areas with a confining layer, where lateral flows dominate vertical flows (Lang et al., 2013). Other topographic metrics such as relief and curvature may also help predict extent and frequency of inundation. Further testing of these metrics in different landscapes is needed to determine how they can best be incorporated into wetland planning (Lang et al., 2013).

2.1.1.3.2 Better Use of Soil Data The SSURGO database provides information on a range of soil hydrologic properties to a depth of approximately 2 m. Wetland restoration planners often rely on hydric soils maps, but data on soil texture, hydraulic conductivity, available water capacity, hydrologic group, drainage class, bulk density, and organic matter are also available through SSURGO. These attributes may account for spatial and temporal variability in soil moisture that is not captured by topographic indices, and help planners predict hydrologic connectivity across the landscape. For example, drainage class can indicate the duration and seasonality of saturation in potential wetland sites. Textural differences in soil layers may indicate preferential flow mechanisms that have important implications for solute transport (Steenhuis et al., 1998).

Although SSURGO currently provides the most detailed soil geographic data in the US, its applications in land management planning are limited by its coarse scale and the spatial aggregation of soil components. SSURGO data consists of polygons representing map units and tabular soil property data associated with distinct components within each map unit. A single SSURGO map unit may contain several major and minor components, each associated with different soils with contrasting properties.

The recent development of gSSURGO, a raster soil dataset prepared by merging vector-based SSURGO data and tabular data, will make it easier for

conservation planners to map these attributes and combine soil information with other datasets. The potential wetland soil landscapes (PWSL) raster layer is an excellent example of the utility of this new dataset (Figure 6). The PWSL expands on hydric soil rankings by identifying soils that were historically hydric based on additional soil attributes. For example, if a pixel falls within a map unit in which the dominant component is not identified as being hydric, the pixel is classified as PWSL if the drainage class is "poorly drained" or "very poorly drained" or the map unit name contains the phrase "ditched" or "drained" (Soil Survey Staff, 2014a). This dataset could provide a basis for identifying sites where wetland hydrology can most readily be restored. PWSL data could be combined with topographic, stream, and land use data to further improve characterization of hydrology and factors contributing to nitrate loss.

Another promising development in soil mapping is the disaggregation of soil map units into probabilistic raster maps of individual components. The distribution of components can be estimated based on correlations with environmental variables such as terrain position, parent materials, topography, and other landscape factors (Nauman and Thompson, 2014; Subburayalu et al., 2014). For example, Nauman and Thompson (2014) queried geomorphic and hillslope profile descriptors in the SSURGO database and developed rules based on environmental raster values for the component landform descriptions for a 3877 km^2 study area in the Appalachian Mountains of West Virginia. These relationships were then used to build a set of training areas for all components, which were used in classification trees with

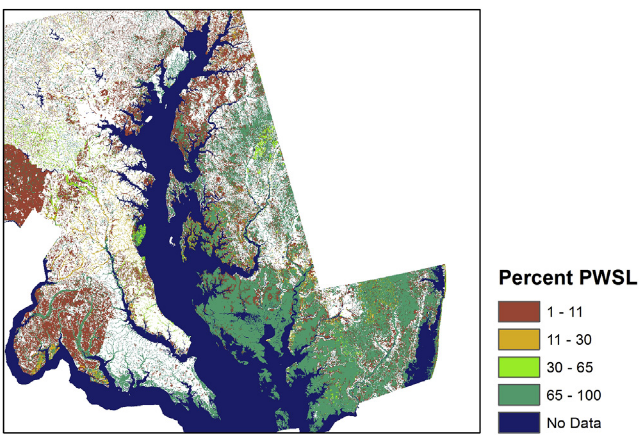

Figure 6 gSSURGO potential wetland soil landscapes (PWSL) (Soil Survey Staff, 2014a).

additional environmental rasters to transform the original SSURGO soil map into a gridded soil component map (Figure 7). Disaggregation has the potential to provide land use planners with more detailed soil maps that better represent spatial variation in soil attributes while reducing the amount of work required to use soil survey data in conservation planning. A similar approach could be developed using environmental rasters such as LiDAR-derived topographic metrics to identify historic wetlands where the hydrology can most readily be restored.

2.1.1.3.3 Incorporation of Ditch Network Data Ditch network data can help identify areas promoting rapid transport of nitrate to streams by ditches. The Eastern Shore Regional GIS Cooperative has digitized tax ditches maintained by public drainage associations in select watersheds, and the data are available for free download (Eastern Shore Regional GIS Cooperative, 2004). As demonstrated by Schmidt et al. (2007), these deeper ditches receive continuous subsurface inputs from upland agricultural fields and can be important conduits for the accelerated delivery of nitrate to surface waters. Field ditches have also been digitized in several watersheds as part of Chesapeake Conservancy's effort to advance the use of geospatial technologies in conservation planning across the Chesapeake Bay watershed (Chesapeake Conservancy, 2014).

2.1.1.3.4 Incorporation of Remote- and Ground-Based Sensor Techniques for Measuring Variability in Soil and Vegetation Characteristics Remote sensing offers a rapid, cost-effective way to incorporate spatial and temporal information on landscape attributes into wetland

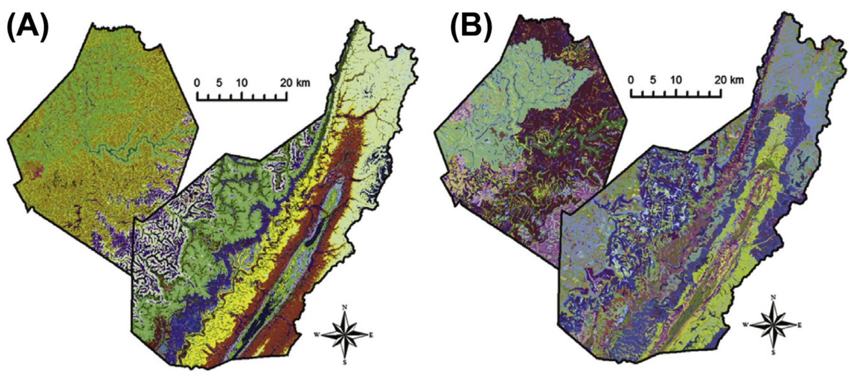

Figure 7 Maps comparing disaggregated SSURGO product (A) with original soil survey map units (B). *Reprinted with permission, Nauman and Thompson (2014).*

restoration planning. Advances in technology and new interpretation techniques have led to improvements in wetland mapping and detection of changes in hydrology, vegetation, and other surface characteristics (Lang et al., 2008; Klemas, 2011, 2013). Although limited primarily to studying changes in surface characteristics, remote sensing has great potential to assist conservation planners in restoration targeting. Airborne and high-resolution satellite imagers may be useful in siting and monitoring small wetlands. Satellite sensors such as IKONOS and QuickBird can provide resolutions of 0.5–1 m in panchromatic bands and 2–4 m in multispectral bands in the visible and near-infrared regions of the electromagnetic spectrum (Klemas, 2011). Color infrared aerial photography and multispectral and hyperspectral imagery may be useful in studying vegetation characteristics that can be related back to wetland function (Klemas, 2013; Tuxen et al., 2008). Indices such as the normalized difference vegetation index, which uses the red and near-infrared bands to detect the spectral characteristics of green plants, can be used to map spatial patterns in crop biomass (Hively et al., 2009). Patterns in crop biomass during dry and wet years may be good indicators of the hydrology of historic wetlands.

Multitemporal synthetic aperture radar (SAR) is particularly promising for wetland studies. SAR microwave energy is sensitive to variations in soil moisture and can pass through vegetation with little attenuation (Klemas, 2013). Lang et al. (2008) demonstrated how multitemporal C-band SAR can be used to detect seasonal changes in hydrologic characteristics of forested wetlands. SAR data are potentially useful for monitoring temporal fluctuations in soil moisture and wetland inundation on prior converted cropland (former wetlands drained for agricultural production). These data could also help us understand the role of forested wetlands upgradient of cropland in diluting nitrate concentrations in local streams.

At field scales, geophysical tools such as ground penetrating radar (GPR) and electromagnetic induction (EMI) can be used to map soil physical properties. Gish et al. (2002) used GPR-derived subsurface digital elevation models (DEMs) to identify preferential flow pathways based on depth to a subsurface clay layer. Subsurface restricting layers within 2 m of the surface benefited corn grain yields during a drought year. Yields decreased with increasing horizontal distance from the GPR-identified flow pathways.

GPR is often used in combination with EMI, which measures soil electrical conductivity (ECa). ECa is affected by factors such as soil moisture, clay content, and mineralogy, which can be used to differentiate soil components (Zhu et al., 2013). Recent studies have explored the application of these

technologies in combination with in-field measurements to evaluate the effects of agricultural practices on soil properties (Jonard et al., 2013) and advance site-specific management for precision agriculture (Zhu et al., 2013). Using similar methods, geophysical tools could be used to evaluate potential restoration sites on prior-converted cropland. Testing of these technologies in different landscape settings is needed to better understand how to use these tools.

Continued advances in geospatial technology and remote- and ground-based sensing will likely improve our ability to predict where in the landscape restoring the hydrology of historic wetlands will promote denitrification or nitrate dilution. Integration of local knowledge with geospatial tools may provide for the most robust analysis of landscapes.

2.2 Estimating Wetland Efficiencies

Wetland efficiencies use simple relationships to predict N removal rates. N removal efficiencies allow conservation planners to make estimates of N attenuation at the watershed scale based on a few selected parameters that are readily available. Efficiencies also allow for directly comparing different N management options to select the most cost-effective choice in terms of dollars per kilogram of nitrogen removed, facilitating policy making and documentation of progress toward achieving TMDLs.

Wetland efficiencies for N are difficult to obtain for a number of reasons. First, nitrate removal efficiencies reported in the literature are highly variable, ranging from negative efficiencies (export of nitrate from the wetland) to greater than 90% nitrate removal (O'Geen et al., 2010). Efficiencies vary with wetland characteristics, climate, landscape position, N loading rates, objectives of restoration, and other factors (O'Geen et al., 2010). Despite the vast number of studies done on the use of wetlands for nutrient treatment, little research has been done on wetlands treating agricultural nonpoint source pollution, and only a handful of studies have been done in the Chesapeake Bay watershed. Efficiency estimates are often based on individual wetlands under highly managed conditions. Whether significant N removal can be achieved at the watershed scale has not yet been tested in the Chesapeake. For modeling purposes, the ratio of wetland to watershed area is commonly used as a surrogate for hydrologic retention time in estimating efficiencies due to the correlation between these variables, but this approach ignores site-specific conditions that can affect N removal, such as the amount of carbon available for denitrification, the permeability of upland and wetland sediments, and local subsurface connectivity.

The challenge of estimating efficiencies at the watershed scale is exemplified by the Chesapeake Bay Watershed Model. Wetland BMPs are assigned efficiencies based on a relationship between percent N removal and wetland area as a percentage of the contributing watershed. Efficiencies are based on geomorphic province, with the assumption that the wetland to watershed area ratio increases moving from upland to lowland regions (Simpson and Weammert, 2009). Wetland BMPs in the Coastal Plain are assigned an efficiency rating of 25%, based on a 4% wetland to watershed area ratio; in the Piedmont Plateau a rating of 14% based on a 2% ratio; and in the Appalachian Plateaus a rating of 7% based on a 1% ratio. These efficiencies were derived through regression analysis of data from 16 studies, only one of which was conducted in the Chesapeake Bay watershed. The model, based on first-order kinetics, fits the data only weakly (Figure 8) and does not capture other factors affecting efficiencies such as wetland age, seasonal variation, flow variability, landscape position, land use conversion, and sediment accumulation (Simpson and Weammert, 2009). When developed, it was expected that these efficiencies would continue to be refined, but without monitoring programs in place to assess wetland performance under different conditions, we have few regional or local data for developing and validating efficiency models.

A second challenge to estimating wetland N removal efficiency is accounting for seasonality of nutrient discharges. Depending on climate and geomorphic setting, the volume and timing of runoff will vary throughout the year. N loss may be event-driven (Kovacic et al., 2000) or it may be attributed to baseflow (Ator and Denver, 2012), warranting different wetland designs and efficiencies. For example, in constructed wetlands receiving tile drainage on the Embarras River in Illinois, the greatest tile discharges and nutrient fluxes occur during pulse flows in the spring and winter seasons (Kovacic et al., 2000). By contrast, in depressional wetlands in the Choptank watershed in Maryland, N fluxes depend on the depth and gradient of the water table, which vary seasonally and are controlled by a complex set of geologic, geochemical, and hydrologic conditions (Denver et al., 2014). In general, N loading in wetlands is likely to be lowest in the summer when crop uptake and microbial activity are greatest. In depressional wetlands on the Mid-Atlantic Coastal Plain, N removal efficiencies will be the greatest when the groundwater flows consistently from the agricultural upland to the wetland and passes through reducing soils within the wetland. With a better understanding of seasonal variability in nutrient

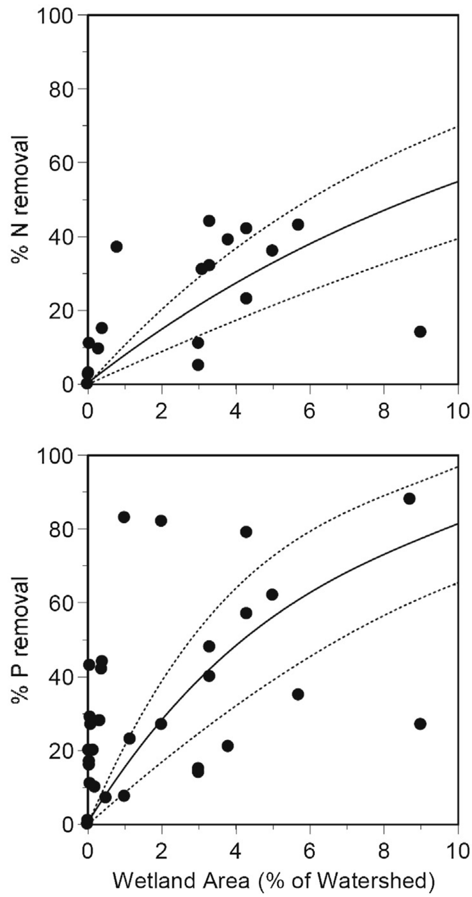

Figure 8 Percentage of nutrients removed annually versus wetland/watershed ratio. *Reprinted with permission, Simpson and Weammert (2009).*

fluxes in wetlands in different settings, a seasonal correction factor may help improve efficiency estimates (Simpson and Weammert, 2009).

Finally, diverse wetland types and approaches to restoration will lead to variability in N removal efficiencies. On the Mid-Atlantic Coastal Plain, denitrification potential varies with wetland hydrogeomorphic type (riverine, depressional, flat) (Jordan et al., 2007). Method of restoration/creation may impact how quickly water quality functions are achieved. For example, ditch plugging causes little disturbance to the soil, but may divert nitrate-rich groundwater to a new groundwater flow path (T. Jordan, 2013, pers. comm.). Scraping, by contrast, removes organic-rich surface

horizons, exposing subsurface horizons. After scraping, some of the topsoil is often replaced, or imported materials such as sand, straw, or compost are added. Restoration methods will likely affect the amount of carbon available for denitrification and the degree of surface-groundwater interactions.

2.2.1 Proposed Approach

Nitrogen removal is most affected by hydraulic loading rate, residence time, nitrate concentration, nitrate loading rate (Crumpton et al., 2006), and carbon availability. A better understanding of how these factors and wetland design affect wetland performance in different hydrogeomorphic settings would help us to develop improved N removal efficiency estimates. As discussed previously, accounting for subsurface flows to, within, and from wetlands is a challenge on the Mid-Atlantic Coastal Plain. We recommend that developing reliable estimates of hydraulic and nitrate loading rates be a priority moving forward. Selective monitoring of wetlands representing a range of environmental conditions would provide documentation of nitrate reduction and data to calibrate models of N removal in wetlands. This is similar to the approach being taken in Iowa to advance watershed-based wetland restoration and construction for water quality improvement in the Mississippi River Basin (Crumpton et al., 2006) (see following section).

3. POLITICAL, SOCIAL, AND ECONOMIC CHALLENGES

In addition to biological and physical challenges, there are a number of political, social, and economic challenges to using wetlands to improve water quality in agricultural watersheds. These include (1) limited information on current wetland practices, (2) broad/unclear objectives of wetland BMPs, and (3) factors limiting landowner willingness to adopt wetland BMPs. Within these challenges, there are questions such as

- How well do existing institutions support wetland targeting for N attenuation?
- How can we ensure that wetlands are maintained so that they continue to provide the expected benefits?
- How do wetlands fit within the broader response strategy for reducing nutrient loads in the Chesapeake Bay watershed?

3.1 Limited Information on Current Wetland Practices

Wetland practices are supported largely by USDA Farm Bill programs that provide technical and/or financial support to landowners. These voluntary

cost-share programs include the Conservation Reserve Program (CRP) and Conservation Reserve Enhancement Program (CREP), the Environmental Quality Incentives Program (EQIP), the Wetland Reserve Program (WRP), and the Wildlife Habitat Incentives Program (WHIP) (Table 1) with the majority of wetland restorations implemented under CRP/CREP and WRP (De Steven and Lowrance, 2011). Wetland projects are also supported by regional and state programs such as the Chesapeake and Atlantic Coastal Bays Trust Fund, Chesapeake Bay Trust, and the National Fish and Wildlife Foundation's Chesapeake Bay Stewardship Fund.

In 2003, the USDA initiated the Conservation Effects Assessment Project (CEAP) to quantify the benefits of conservation practices implemented under the Farm Bill. CEAP includes a wetland component aimed at assessing the effectiveness of wetland practices in providing ecosystem services through several regional and watershed-scale studies (Brinson and Eckles, 2011). The CEAP Wetlands Mid-Atlantic study has been underway since 2008 with data collection ongoing on restored and natural wetlands and prior converted cropland in Delaware, Virginia, and Maryland. CEAP's effort has been limited in the Piedmont and North Atlantic Coastal Plain by a lack of information on how conservation practices are implemented in the field (De Steven and Lowrance, 2011). Farm Bill conservation programs do not require monitoring of conservation benefits, and since projects are carried out on private land and farmer privacy is protected

Table 1 Farm bill conservation programs

Program	Incentive type	Contract period (year)
Conservation Reserve Program (CRP)	Annual rents plus cost share	10–15
Conservation Reserve Enhancement Program (CREP)	Annual rents plus cost share, easements	10–15
Wetland Reserve Program (WRP)	Cost share or one-time easement plus cost share	10 year contract; 30 year/permanent easement
Environmental Quality Incentives Program (EQIP)	Cost share	1–10
Wildlife Habitat Incentives Program (WHIP)	Cost share	1–10 and 15+

under Section 1619 of the Food Security Act, opportunities for research have been limited prior to CEAP. Detailed spatial data on wetland practices would allow us to more fully quantify the effects of wetlands on water quality at watershed scales (Gleason et al., 2011), but this information is not publicly available for most programs (WRP easements are the exception). Information on Farm Bill expenditures on these practices, beyond overall program expenditures, is not reported either (USDA Natural Resource Conservation Service, 2013). Expenditures on wetland practices could help us evaluate the cost-effectiveness of these BMPs at county, state, and regional scales.

3.1.1 Proposed Approach

We recommend that monitoring plans be built into conservation programs. Following the recommendations of CEAP, monitoring programs should (1) specifically evaluate response to treatment (i.e., nitrate reduction through wetland treatment), (2) monitor conservation practices as intensively as water quality (i.e., through documentation of wetland restoration methods and maintenance), and (3) invest in long-term monitoring and technical expertise (Osmond et al., 2012). A coordinated monitoring program could be conducted on a representative subset of wetlands, across a range of geomorphic and climatic settings. Measurements of inflow and outflow rates, nitrate concentrations, and temperature could be collected regularly throughout the year to account for seasonal variability in nitrate removal. With time, these measurements could be used to calibrate models of wetland performance in different landscapes. The monitoring program employed by Iowa CREP (see Box 1) may serve as a role model for the Chesapeake Bay watershed. Alternatively, a program could be established whereby landowners monitor wetlands themselves, with appropriate technical oversight by the Natural Resources Conservation Service (NRCS) or partner organizations to ensure data quality. To our knowledge, this has not been tested, but it is possible that landowners would be willing to have wetlands on their property monitored so that they could document reductions for their nutrient management plans.

In addition to a coordinated monitoring program, we propose that standards for recordkeeping be established to compare siting and design methods. At a minimum, prerestoration conditions (soils, hydrology, vegetation), methods of restoring hydrology, degree of earth-moving, addition of imported materials, and actual costs should be recorded in order to better evaluate practices and make future recommendations.

Box 1 Iowa conservation reserve enhancement program

The Iowa Conservation Reserve Enhancement Program (CREP) is an excellent example of how water quality monitoring can be built into a wetland conservation program. These CREP wetlands are strategically located and designed to remove nitrate from tile-drained cropland (Iowa Department of Agriculture and Land Stewardship, 2013). Representative wetlands are monitored each year to document nitrate reduction (Crumpton et al., 2006). Wetlands selected for monitoring range from 0.5% to 2% wetland/watershed ratio and 10 to 30 mgl^{-1} average input nitrate concentrations (Crumpton et al., 2006). Weekly grab samples are taken from each wetland, and automated samplers and flow meters are installed at inflows and outflows at a subset of wetlands. In addition, water levels are monitored continuously at outflow structures and water temperature is continuously recorded. In support of the CREP monitoring program, mass balance analysis and modeling are used to estimate variability in performance of CREP wetlands based on temperature and precipitation patterns (Crumpton et al., 2006). These CREP wetlands reportedly remove 40% to 90% of nitrate inputs (Iowa Department of Agriculture and Land Stewardship, 2012).

Finally, we suggest that program expenditures be reported along with enrollment by acreage and count. Expenditure data would help us assess how programs are allocating their funds, and compare how much funding is available for wetland restoration/creation with how much is spent. Expenditure data would also allow for better accounting of the cost of wetland practices and help planners determine which programs are most cost-effective with regard to implementing wetland BMPs for water quality improvement.

3.2 Broad/Unclear Objectives of Wetland BMPs

A number of wetland practices are available through WRP, CRP, EQIP, and WHIP, four of which are considered water quality BMPs: wetland construction, wetland restoration, wetland creation, and wetland enhancement (Table 2). The emphasis of these practices has traditionally been on provision of waterfowl and wildlife habitat, with little attention given to water quality in siting and design (Crumpton et al., 2006; De Steven and Gramling, 2012). Wetland construction (656), the only Farm Bill wetland practice whose explicit purpose is to reduce agricultural runoff, is not yet an approved practice in any of the Bay states, although the Maryland NRCS is currently considering adding it to the list of approved CREP practices (S. Strano, 2012, pers. comm.).

Table 2 Wetland conservation practice standards

NRCS Conservation Practice Standard	Purpose
Constructed wetland (656)	To reduce pollution potential of runoff and wastewater from agricultural lands to water resources
Wetland restoration (657)	To restore wetland function, value, habitat, diversity, and capacity to a close approximation of the predisturbance conditions by restoring conditions conducive to hydric soil maintenance, wetland hydrology, native hydrophytic vegetation, original fish and wildlife habitats
Wetland creation (658)	To establish wetland hydrology, vegetation, and wildlife habitat functions on soils capable of supporting those functions
Wetland enhancement (659)	To increase capacity of specific wetland functions by enhancing hydric soil functions, hydrology, vegetation, enhancing plant and animal habitats

The degree to which water quality is addressed in restoration depends on the program and on the priorities of local and state governments. CREP program guidelines limit enrollment to eligible cropland containing prior-converted and farmed wetlands, while the WRP allows eligibility of hydrologically degraded wetlands on rangeland and forest production lands as well. In Maryland, WRP projects often consist of plugging ditches on forested land that does not receive agricultural N. These restorations remove little, if any, N from upland areas, although they may help improve regional water quality through dilution with low-nitrate water (Denver et al., 2014).

Priorities of local conservancies and wildlife organizations also direct wetland restoration objectives. For example, Ducks Unlimited has frequently partnered with the US Fish and Wildlife Service and local agencies to restore wetlands, with the objective of creating waterfowl habitat (Ducks Unlimited, 2014). The Nature Conservancy (TNC) is actively involved in wetland restorations, working with federal and local agencies and landowners to carry out targeted restoration efforts to improve water quality and wildlife habitat (The Nature Conservancy, 2014). TNC has developed a LiDAR-based targeting tool to site wetlands where they can intercept nutrient and sediment runoff (The Nature Conservancy, 2013). By working with scientists, conservation, planners, and other stakeholders, these efforts can help direct conservation program resources toward

projects that have greater potential to achieve improvements in water quality.

Several of the state WIPs include specific levels of wetland restoration by 2025 to help meet the Chesapeake Bay TMDL, including Maryland (6000 ha), Virginia (7776 ha), Delaware (2317 ha), New York (5581 ha), Pennsylvania (21,908 ha), and West Virginia (164 ha) (D. Hopkins, 2014, pers. comm.). WIPS are developed in consultation with local partners at the county scale (Maryland Department of the Environment, 2012); so planned wetland acreage should represent the combined amounts of individual county wetland goals. It is not clear how WIP planners arrived at these acreage goals and whether all of these projects include water quality as an explicit objective. For example, some WRP projects may be included that are not situated to receive significant agricultural runoff (USDA NRCS, 2012, pers. comm.; MDE, 2012, pers. comm.). Most wetland conservation practices have broad objectives, and water quality improvement is often an assumed benefit of restoring wetland hydrology, rather than an explicit objective. Restoration calls for the "return of a wetland and its functions to a close approximation of its original condition as it existed prior to disturbance" (USDA Natural Resource Conservation Service, 2014). It may be unrealistic to expect though that in working agricultural landscapes, we can recreate historic wetland conditions (Zedler, 2003). Prioritizing nutrient removal may conflict with other wetland functions, such as provision of wildlife habitat (Brinson and Eckles, 2011). For example, wetlands receiving high N and P inputs can become dominated by monocultures of *Typha* spp. or similarly aggressive plant species (Woo and Zedler, 2002). Thus, establishing objectives and evaluating wetland success will require consideration of the multiple services wetlands provide and balancing the demands of the TMDL with additional local, state, and regional priorities.

3.2.1 Proposed Approach

Wetlands provide a number of ecosystem services, including filtering nutrients and sediments, providing wildlife habitat, flood control, and carbon sequestration—all of which are valuable restoration outcomes but may not all be achievable in any given project. The WIPs are intended to document how Bay jurisdictions will achieve nutrient reductions needed to meet the TMDL. We propose, therefore, that wetland restorations credited in WIPs include the explicit objective of improving water quality in project siting and design.

Programs that seek to reduce nonpoint source pollution, such as CREP, may be best suited for implementing these restorations. Alternatively, Bay states could issue a directive establishing that WRP, CRP, and other wetland projects that are credited toward WIP wetland acreage include water quality as an objective. Performance-based evaluation through monitoring of select projects would add value by enabling Bay jurisdictions to document nutrient reductions, develop estimates of efficiency for different geomorphic and hydrologic settings, and strategize placement of wetlands.

3.3 Landowner Willingness to Adopt

Farm Bill programs are voluntary, with landowners typically approaching local soil conservation districts to get support for implementing conservation practices. Some programs, such as the Virginia CREP, have used a targeting approach to direct outreach efforts toward landowners with eligible acreage (E. Horsley, 2013, pers. comm.). One of the greatest challenges moving forward with a watershed approach to wetland restoration will be the degree to which landowners are willing to adopt these practices. Possible obstacles to landowner participation need to be explored in order to develop educational programs on wetlands and water quality and direct outreach efforts toward those people most likely to adopt practices (David et al., 2013). Although no systematic study of farmer attitudes toward wetlands has been conducted in the Chesapeake Bay watershed, reports from other regions as well as research on agricultural BMP adoption provide insight into farmers' perceived costs of wetlands and factors that might impede adoption.

A recent study in Sweden identified "land management in the best possible way" as the primary motive of farmers considering constructing a wetland on their land (Hansson et al., 2012). Farmers surveyed in this study viewed food production as the ultimate use of the land, and thought productive land should be kept in cultivation. Land that is unproductive or marginally productive could be considered for other income-generating activities. In the US, high commodity prices incentivize farmers to plant on all arable land, including land with poor drainage where crop success is highly variable year to year. Farmers in Kansas reported wetland areas can be harvested three years out of five with only slightly below average productivity (Gelso et al., 2008). In the Mid-Atlantic Coastal Plain, in a dry year the wetter areas—areas where wetlands would be targeted—are often the farmers' most productive land. The challenge, therefore, is to identify the value farmers place on these areas.

- How does the value vary with frequency and duration of saturation?
- Under what conditions are these lands considered marginal or unproductive?

Producers see themselves as stewards of the land, but economic and other objectives may outweigh stewardship goals (David et al., 2013). Farmers must consider their decision to adopt a given BMP within the context of their entire farm operation (David et al., 2013). Meeting the needs of landowners may limit options for wetland siting and design. However, in some instances, it may be desirable to take "productive" land out of production to achieve water quality benefits. It may be necessary to expand the concept farmers have of land productivity to include ecosystem services other than food production, as recommended by Hansson et al. (2012).

Several other deterrents to wetland restoration can make obtaining landowner cooperation difficult. Gelso et al. (2008) found that a high degree of wetland dispersion on the farm substantially increases the perceived costs associated with wetlands, indicating that farmers are inconvenienced by having to transport equipment around wetland areas. These "inconvenience costs" limit options for siting wetlands at the farm level. For example, the best place to site a wetland to capture nitrate might be in the middle of the field, but the farmer may only be willing to put in a wetland at the edge of the field where it will not be in the way of farm operations.

A related issue is wetland maintenance. The effectiveness of wetlands in improving water quality often depends on the degree to which the wetlands are maintained for this purpose (Seitzinger et al., 2006). A long-term view is implicit in a watershed-scale approach, and requires consideration of both the ecological and programmatic lifetimes of conservation practices (Brinson and Eckles, 2011). For wetlands receiving high sediment loads, the ecological lifetime may be particularly short due to loss of surface water storage capacity through sediment infilling (Brinson and Eckles, 2011). A possible solution would be to periodically excavate the wetland, but this may impose additional inconvenience costs on the landowner.

An additional concern shared by many farmers is the possibility of negatively impacting the drainage rights of their neighbors. Maintaining good relations with neighbors can be a priority value among farmers. Uncertainty about the effects of plugging a ditch or otherwise altering drainage on ditch networks may discourage farmers from installing wetlands. This relates to the larger issue of farmers' understanding of the effects of wetland restoration on hydrology and local and regional water quality.

Farmers may not understand how wetlands contribute to nutrient removal at the farm and watershed scale (David et al., 2013; Hansson et al., 2012). Hansson et al. (2012) reported that interest in wetlands was lower among farmers who knew less about wetland ecosystem services. The traditional focus on the wildlife benefits of wetlands in US conservation programs indicates that farmers may appreciate the wildlife values, and are often persuaded by the hunting opportunities wetlands provide. The water quality benefits are less obvious, particularly since they are so rarely documented. Producers cannot see the loss of nutrients and may feel disconnected from the downstream effects (David et al., 2013). In the Mississippi River Basin, farmers' growing mistrust of policy makers is also a major barrier to collaboration (David et al., 2013). On the other hand, acknowledgment that a constructed wetland is in fact contributing to nutrient reduction can give farmers a more positive feeling about wetlands, and even a sense of pride and satisfaction (Hansson et al., 2012). This finding provides further justification for the need for a coordinated monitoring program.

3.3.1 Proposed Approach

Studies on farmer attitudes in the Chesapeake Bay watershed toward wetlands would help us identify possible barriers to implementing a watershed-scale approach to wetland restoration. Results could be used to target practices that meet the needs of landowners and compare different N management strategies. A monitoring program also has the potential to help us meet this challenge. By directly linking water quality benefits to wetland conservation practices, farmers could document nutrient reductions in their farm operations. Assigning a dollar value to units of nutrients removed through performance-based incentive payments or nutrient trading programs could enhance the perception that wetlands are "productive" and even profitable.

4. CONCLUSIONS

Due to the large percentage of land in agriculture and the extent of subsurface drainage, the Mid-Atlantic Coastal Plain is an appealing choice for wetland restoration and creation in the Chesapeake Bay watershed. While the opportunities to restore wetlands in this region are abundant, there are numerous challenges to locating and designing wetlands to capture nitrate runoff. Due to the heterogeneity of the surficial aquifer, variability in soil hydrologic characteristics, and seasonality of hydrologic connections, accounting for subsurface connectivity between nitrogen sources and wetlands is a

challenge. Social, political, and economic constraints further complicate using wetlands to reduce nonpoint source pollution. There are a number of steps we can take to improve the likelihood that wetlands will contribute to water quality goals. Information on subsurface connectivity between nitrogen sources and wetlands is a significant challenge. We believe that this challenge can be addressed through improved assessment of hydrologic connectivity in areas with artificial drainage; conducting catchment-scale studies of hydrogeomorphic predictions of hydrologic connectivity; and improved use of geospatial data for predicting subsurface connectivity between N sources and wetlands including LiDAR, soil survey, ditch network data, and remote- and ground-based sensing techniques. Our poor ability to estimate wetland efficiencies can be addressed by implementing a coordinated monitoring program to assess the success of these projects across environmental conditions and management practices. Such a monitoring program would also provide needed information on the implementation of wetland practices supported through government programs. The use of programmatic information would also be improved with better recordkeeping standards and the reporting of expenditures, enrollment, acreage and count within these programs. Requiring water quality to be an explicit objective of restorations included within WIP accounting would avoid the inclusion of projects with minimal water quality benefits. Finally, we believe that research is needed on farmer attitudes in the Chesapeake Bay watershed toward wetlands for water quality protection.

Scale will be an important consideration moving forward with a targeting approach. State WIPs are developed at the county scale, but watersheds may cover multiple counties. At the scale of the entire Mid-Atlantic Coastal Plain, it may be useful to allocate efforts according to hydrogeomorphic region, with more effort to promote wetland BMPs in the "poorly drained uplands" and "surficial confined" regions. For local watersheds the size of a few thousand hectares, we believe that partnerships between government agencies, conservation planners, and researchers will facilitate engagement of landowners and selection of appropriate N management strategies. High resolution GIS data and tools will be important components of the planning process. At field scales, siting and designing wetlands with careful consideration of hydrogeomorphic controls on nitrate removal and integration of wetland BMPs into farm operations is critical.

Wetland BMPs are just one approach to addressing water quality, and must be considered in the context of the entire suite of agricultural BMPs that can be used to mitigate nonpoint source pollution. In addition to edge-of-field and off-site practices, changes in management practices to

reduce N inputs will also be needed to help meet N reduction goals. By advancing our understanding of nitrate transport to potential wetlands on the coastal plain and working collaboratively with landowners, we can target areas where we expect to find the greatest benefits through wetland restoration and creation practices.

Wetlands can provide multiple ecosystem services and be an integral part of conservation programs on the Mid-Atlantic Coastal Plain. The demands of the TMDL will need to be balanced with these multiple objectives. Moving forward, we believe our proposed actions would clarify and support the use of wetland restoration and creation practices to meet water quality goals.

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CHAPTER TWO

Nitrogenous Gas Emissions from Soils and Greenhouse Gas Effects

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Abstract

The Haber—Bosch process for synthesizing fertilizer nitrogen (N) is among the most important modern discoveries because it has enabled us to grow enough food for several billion more of us. At the same time, however, profusion of added "reactive" N has become a prominent ecological threat, globally, because a large fraction of applied N is lost from agricultural ecosystems. Nitrogen added to agricultural soils, in organic forms or as synthetic N fertilizers, has one of four fates; it can be assimilated by plants, lost to surface- or groundwater, retained in the soil, or lost to the atmosphere. The last of these is particularly worrisome because of links to climate change and other threats to the biosphere. Our aim is to summarize briefly the processes of atmospheric N emissions to the air from agricultural ecosystems, and to consider how management practices might reduce those emissions. Nitrogen gases emitted from soil emanate from naturally occurring biological processes regulated largely by three interactive factors: substrate availability, aeration, and temperature. Although these factors are partly dictated by weather and intrinsic soil properties, they are also influenced by management so that emissions can be heavily influenced by practices imposed on the land. Variables to consider in devising systems with reduced emissions include: forms, rate, and timing of fertilizer; tillage and residue management; crop rotation, including the use of legumes; and manuring practices. All of these need to be considered together to devise systems, tuned to local conditions, which not only reduce emissions but also meet growing demands for agricultural yields. Developing such systems, based on holistic understanding from many disciplines, is now critical to sustain the long-term productivity and vitality of our ecosystems.

1. OVERVIEW OF NITROGEN CYCLE

If you ask 'what has been the most important invention of the past 100, 150 years?' it's been the synthesis of ammonia. If we could not synthesize ammonia by taking nitrogen from the air, hydrogen from natural gas and pressing them together in the Haber—Bosch cycle… if we could not do this to make nitrogen fertilizers, we could not grow enough food for about 40% of people. So you are talking about something like three billion people. In existential terms, that is the most important invention.

Vaclav Smil

Nitrogen is one of the most important elements to all life on earth, including that of humans. It is abundant in the atmosphere, yet deficient

for living things in most terrestrial ecosystems, because atmospheric nitrogen (N) occurs mostly as dinitrogen (N_2), a molecule containing two N atoms with a triple bond between them. Only when this strong bond is severed can the N become reactive and available for use by organisms.

In the early 1900s Haber and Bosch devised a practical industrial way of converting N_2 to reactive N, removing constraints of N deficiency, and vastly increasing agricultural yields (Smil, 2001). Since then N use in agricultural systems has increased, as has food production, but especially so in the last 50–60 years; from 1950 to 2000, fertilizer N applied globally increased 20-fold (Bouwman et al., 2013). In the future, N fertilizers will be critical for producing the large quantities of food needed to feed the burgeoning world population, which is expected to reach more than nine billion in less than 40 years (Zavatarro et al., 2012). But the bioavailability of reactive N that makes it an effective fertilizer also allows it to readily dissipate through the environment and cascade through the atmospheric, aquatic, and terrestrial ecosystems where it can have negative effects on human health, ecosystem services, and climate change (Erisman et al., 2013) (Figure 1). Thus reactive N is both a boon for humanity, and

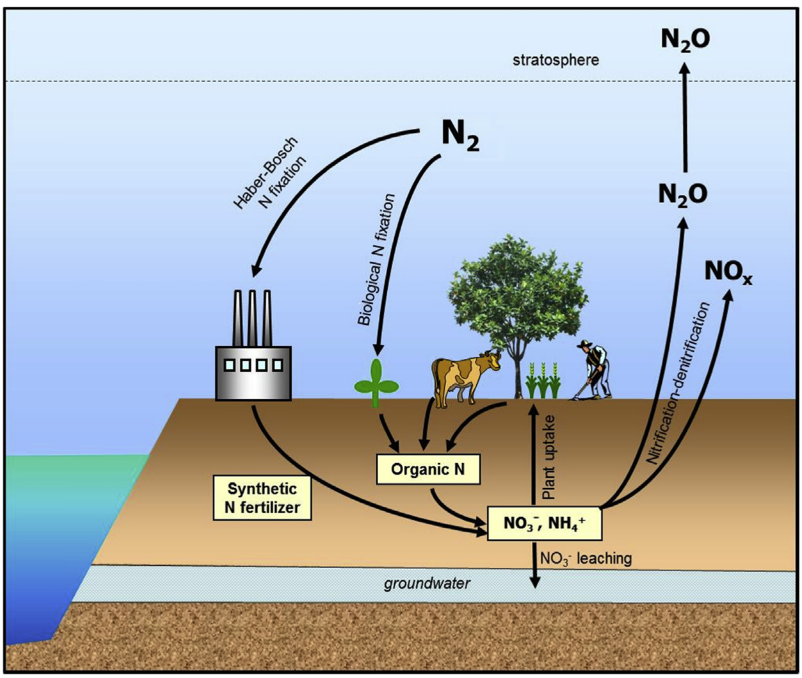

Figure 1 The nitrogen cycle.

one of our most prominent threats to the biosphere. Some have argued that the goals of producing more food with low pollution (dubbed "Mo Fo Lo Po") will not be achieved solely by technological developments, but will also require policies that recognize the economic and social factors affecting decisions made by farmers (Davidson et al., in press).

Our purpose in this chapter is to review gaseous emissions from soil of N compounds contributing to radiative forcing. About 10% of total anthropogenic greenhouse gas emissions originate from agricultural activities (IPCC, 2013), of which N gas emissions (mostly nitrous oxide, N_2O) account for about 60%, largely from reactive N applied to soils (Reay et al., 2012; Smith et al., 2007). We will briefly summarize the contribution of agriculture to atmospheric N gases by describing the forms, sources, and microbial pathways of these gases and discuss how their emission is influenced by agricultural practices. Some mitigation strategies will be presented along with a short discussion of the trade-offs and opportunities involved in managing N in agroecosystems; this is to broaden the perspective and extend the focal plane of how we might best manage N in the future.

2. THE CONTRIBUTION OF AGRICULTURE TO ATMOSPHERIC N GASES (NO_2, NO, N_2O)

Intensive cropping systems that produce large quantities of food, forage, biofuel, and fiber require adequate N, the nutrient that most often limits the rate of net primary production, and attainment of maximum yields. Most of the reactive N applied is fixed industrially, via the Haber–Bosch process, or biologically, via the legume–*Rhizobium* symbiosis (Fowler et al., 2013). Synthetic N fertilizer is the biggest source of N added to intensive cropping systems. Ideally, fertilizer is applied to match temporal plant N demands, but rarely is such synchrony attained because of logistical, economic, and biological contraints, so that a large fraction of this reactive N is typically lost. Large amounts of N are also added in animal manures—globally this amount exceeds that of N fertilizer (Bouwman et al., 2013)—but this N primarily represents recycling of N originally fixed industrially or biologically.

Gaseous N escaping from soil affects radiative forcing directly through the emission of N_2O and indirectly because emissions of N oxides, such as NO_2 and NO (together known as NO_x), affect atmospheric concentrations of other greenhouse gases, especially ozone (O_3) and methane (CH_4). Ammonia emissions from soil (especially soils receiving urea fertilizers or livestock

manure) also contribute to aerosol formation, and eventually may be converted to NO_x or N_2O. The emissions of N_2O come primarily from agricultural soils and contribute to climate warming on short- and long-timescales. In this review, we focus primarily on emissions of N_2O, the dominant nitrogenous greenhouse gas, and NO_x, because of its close biochemical links to N_2O.

Only a small portion (about 0.5–3%) of N applied to cropped soils is emitted as N_2O (Stehfest and Bouwman, 2006; Linquist et al., 2012) but these emissions make a major contribution to the overall greenhouse gas budget (Robertson et al., 2013) because N_2O is such a potent contributor to radiative forcing, with each molecule about 265 times more effective than a molecule of CO_2 in absorbing out-going long-wave solar radiation on a 100-year time frame (IPCC, 2013). The estimated loss of N, as a proportion of that applied, varies widely because of soil variability, interactions with weather, and uncertainty of measurements.

2.1 Atmospheric Fates and Consequences of N Gases

Behavior of N_2O and NO varies among layers of the atmosphere. N_2O is inert in the lower atmosphere (i.e., troposphere) where it has a mean residence time of more than 100 years, but in the upper atmosphere (i.e., stratosphere) it participates in photochemical reactions that destroy ozone (O_3), which absorbs most of the incoming high-frequency UV radiation (Ravishankara et al., 2009). In contrast, NO participates in tropospheric photochemical reaction that produce O_3 and its mean residence time there is too short for it to reach the stratosphere where it might destroy O_3.

In the troposphere NOx is a key factor in the creation of O_3, which reacts with other atmospheric chemicals that affect crop productivity and human health. As a strong oxidant, O_3 can cause severe leaf damage (e.g., chlorosis, necrosis, and lesion formation (Vainonen and Kangäsjarvi, 2014) and interferes with the ability of plants to produce and store carbohydrates (Betzelberger et al., 2012). Continued exposure to O_3 can make sensitive plant species more susceptible to damage from disease, insects, and severe weather (EPA, 2014).

3. FORMS, SOURCES, AND PATHWAYS OF N GASES

Nitrogen enters agricultural soils as unintentional recycling of crop residues and livestock excreta, and as intentional applications of synthetic

N fertilizers and organic by-products (especially livestock manure). Most of the N entering agricultural soils is applied as, or is converted to, soil ammonium, nitrite or nitrate (collectively called plant-available N). Subsequently this N can be, (1) taken up by plants, (2) lost to surface and/or groundwater, (3) retained in the cropping system as soil organic or inorganic N, crop residues, microbial biomass, soil organic matter, or in plant tissues (i.e., in perennial crops), (4) lost to the atmosphere as nitrogen dioxide (NO_2), nitric oxide (NO), nitrous oxide (N_2O), ammonia (NH_3), or N_2. These N gaseous emissions from agricultural systems have been widely studied because of their effects on radiative forcing and their contribution to global warming as greenhouse gases.

Most NO_x produced in cropped soils is formed as intermediates and derived from the same two biological processes (nitrification and denitrification); its emission from soil is therefore governed by the same environmental and agronomic factors. Emission of NO_x in cropped systems is also highly episodic and in some cropped systems the magnitude of NO_x emissions is similar to those of N_2O (e.g., Matson et al., 1998). Global NO_x emissions from cropped and grassland soils, however, are likely less than half of the global N_2O emissions (Stehfest and Bouwman, 2006).

3.1 Biological Nitrification and Denitrification—Physical Factors and Biological Processes

N_2O is formed in soils primarily as a by-product of biological nitrification and as an intermediary step of denitrification. In nitrification, NH_4^+ is oxidized to NO_3^- with NO and N_2O being metabolic by-products released to the atmosphere (Figure 2). Under aerobic conditions, autotrophic nitrification is the main source of N_2O with heterotrophic nitrification playing

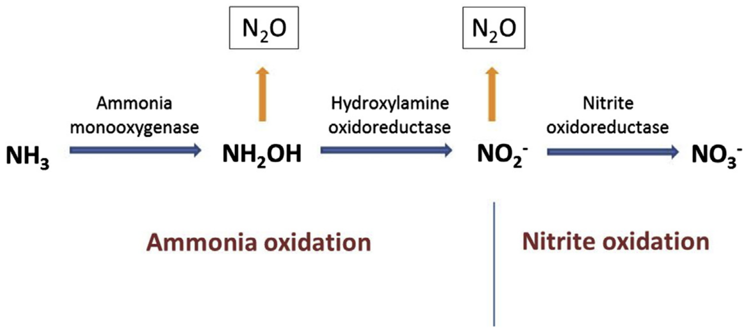

Figure 2 Nitrification.

a minor role. The initial step of nitrification, ammonia oxidation, is performed by both bacteria (AOB) and archaea (AOA). Differences in the specific conditions under which each of these two groups predominates, as well as differences in the catalyzing enzymes involved in the reaction, are thought to differ, affecting the amounts of N_2O produced during the conversion of NH_3 to NO_3^-. Nitrifiers are physiologically diverse leading to a range of soil environmental conditions under which they can function and produce N_2O (Yao et al., 2013; Prosser and Nicol, 2012; Taylor et al., 2012).

In denitrification, NO_3^- is reduced in steps to N_2 and, in the process, NO and N_2O can be formed as intermediates and released to the atmosphere. The stepwise reactions are carried out by denitrifiers, predominantly heterotrophic microorganisms that are facultative anaerobes (Figure 3).

Capacity to perform this process is spread broadly across many taxa of bacteria with as many as 50% of cultured phyla-containing organisms capable of performing at least some steps of denitrification pathway (Prosser, 2007). As facultative anaerobes they cope with suboxic or anoxic conditions by using NO_3^- in place of O_2 as an electron acceptor. The sequence of reactions in denitrification proceeds via the formation of NO, but NO is usually not emitted from wet, anaerobic soils (Kool et al., 2010). The ratio of NO-N/N_2O-N decreases sharply with increasing water-filled pore space, apparently because NO is more rapidly consumed than N_2O by denitrifying microorganisms (Firestone and Davidson, 1989).

Although produced by both nitrifiers and denitrifiers, N_2O is also produced in the process of nitrifier-denitrification, in which NH_3^- is oxidized to NO_2 which in turn is reduced to N_2O and N_2 (Figure 4). This process is carried out by one group of microorganisms, autotrophic NH_3-oxidizing bacteria, under decreasing O_2 concentration and can be an important source of N_2O (Wrage et al., 2001). In contrast, ammonia-oxidizing archaea are not

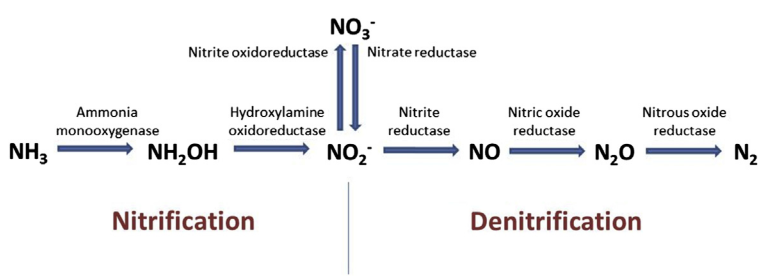

Figure 3 Stepwise reactions involved in denitrification; showing enzymes involved in each step.

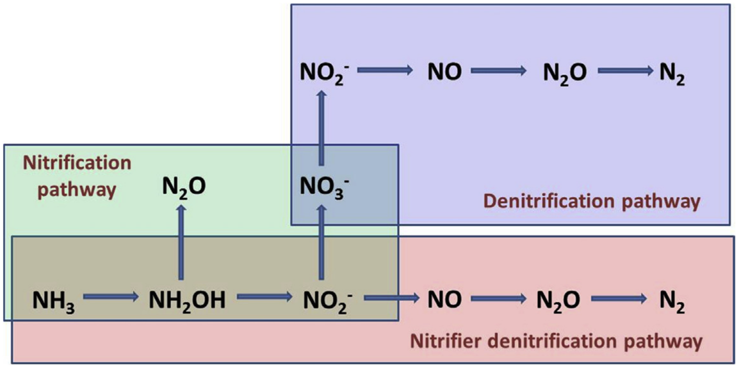

Figure 4 Transformations of mineral N in soil that lead to emission of N_2O and N_2 via different pathways. *Adapted from Wrage et al. (2001).*

believed to perform nitrifier-dentirification (Steiglmeier et al., 2014), limiting their overall contribution to N_2O production. Nitrifier-denitrification thus differs from coupled nitrification-denitrification, in which various groups of coexisting microorganisms transform NH_3^- ultimately to N_2 (Wrage et al., 2001). In some soils (e.g., nutrient-poor, sandy soil) nitrifier-denitrification was a major contributor (up to 25–50%) to N_2O emissions when soil water content conditions are less than optimal for heterotrophic denitrification (Kool et al., 2011; Venterea, 2007).

The O_2 concentration in the soil matrix varies to the extent that there are adjacent pockets of aerobic and anaerobic conditions. The close proximity of these pockets can result in a coupling of nitrification and denitrification processes, i.e., the production of N_2O from nitrification occurring in aerobic zones and N_2O produced by denitrification occurring in the anaerobic zones or by depletion of O_2 by oxidation of NH_4^+ followed by the anaerobic reduction of NO_3^-. But the N_2O may not be detected at the soil surface, because N_2O produced in either of these zones may diffuse to a well-oxygenated zone where it can be reduced further to N_2. The opposite may also occur: the N_2O produced within a saturated anoxic aggregate can be quickly reduced further to N_2. Thus the production and emission of N gases derived from the processes like nitrification, denitrification, and nitrifier-denitrification, depend heavily on the structure and water content of the soil.

Dissimilatory reduction of nitrate to ammonia (DRNA) occurs through intermediary NO_2^-. The reduction of NO_2^- may produce N_2O, but DRNA is often overlooked in process-scale prediction of N_2O emissions (Baggs,

2010). Although DRNA is usually deemed an important process in tropical systems (RÜtting et al., 2008), where C is abundant compared to N and conditions are highly reducing, recent information suggests that it may occur also in temperate arable soils (Schmidt et al., 2011). Both DRNA and denitrification are promoted by anaerobic conditions and C availability, but DRNA conserves N in the system by producing NH_4^+ rather than N_2 which is lost to the atmosphere. Therefore DRNA is particularly important in N-limited systems.

Changes in microbial biomass can affect N_2O emissions by increasing the abundance of N_2O producers and consumers (i.e., the potential for N_2O emission) and conversely by increasing the capacity of organisms that do not produce N_2O to immobilize substrates for nitrification and denitrification (C and N). A change in community composition can also affect N_2O emissions because individuals within these functional groups can differ physiologically and thereby affect the amount of N_2O produced during transformations (Webster et al., 2005; Prosser and Nicol, 2012). In the long-term Broadbalk Wheat Experiment, Clark et al. (2012) observed that the relationship between denitrification fluxes and denitrifier abundance or community composition (*nir*K, *nir*S, *nos*Z) was influenced by management through links with soil C and N. Thus, the interaction of management, soil type, and environment has complex effects on the dynamics of N_2O-producing organisms over wide-ranging scales of time and space that ultimately lead to unpredictable differences in N_2O production.

3.2 Conceptual Models: How Factors, Processes, and Levels Regulate N Gas Emission from Soil Hole-In-Pipe Model

The hole-in-the-pipe model (Firestone and Davidson, 1989) uses an analogy of a leaky pipe in which NO and N_2O are emitted (Figure 5). The rate of flow of N through the pipes is analogous to the rates of nitrification and denitrification. Several factors regulate the flow rate of N through the pipes: the amount of mineral soil N, soil temperature, and soil water content. The NO and N_2O gases leak out of holes in the pipes; the size of which is determined primarily by the water content of the soil. Other factors also control the proportions of NO and N_2O produced; these include mineral N concentrations, pH, and available C. Another set of factors controls the consumption of NO and N_2O within the soil pore space; these include diffusion and mass flow, which in turn are controlled by soil structure, air-filled pore space, and temperature.

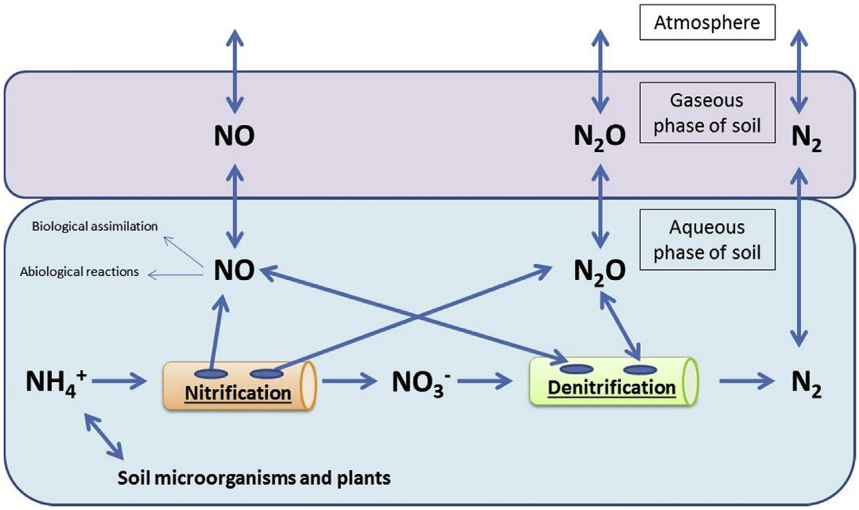

Figure 5 Diagram of hole-in-the-pipe conceptual model. *Adapted from Davidson (2000).*

3.2.1 N Gas Emission as Function of Soil Pore Space Properties

The production and emission of N gases from soil are primarily the result of biological activity, but that N-gas generating activity can be regulated to a large extent by soil structural properties (Ball, 2013). The production and emission of N_2O from soil are regulated by three main factors: substrate availability, aeration status, and temperature (Smith, 1980). These three factors interact and are affected by the timing of weather events and soil physical conditions.

Linn and Doran (1984) proposed a simple model, which illustrates the extent to which water content, expressed as water-filled pore space, controls the production of N_2O and CO_2. In their model, the critical point at which dominant aerobic/anaerobic processes begin to exert control on the production of these gases is at 60% water-filled pore space, which approximates field capacity for most soils with a loam texture. Field capacity is defined as the soil water content after excess water has been drained from the soil; at this water content the soil macropores have drained and are air-filled but the micropores are still water-filled. This breakpoint of 60% water-filled pore space is assumed to be the point of transition at which oxidative and reductive processes are active.

This model is useful for a general understanding of the effects of soil structure, as characterized by water-filled pore space, on N gas emissions

from soil. A more detailed understanding can be obtained by evaluating the size and distribution of soil pores. When a soil is compacted, for example, the volume of pores is reduced. Not all pores are reduced similarly however, because the largest pores are usually lost or compressed first (Richard et al., 2001). This preferential loss of larger pores changes the pore size distribution, thereby affecting relative diffusivity, air permeability, and oxygen content (Ball, 2013), which in turn control the production and emission of N_2O. Thus, the increased denitrification commonly observed in compacted soils may not be attributed only to a reduction in overall soil pore space but more immediately by preferential loss of large pore spaces tied to diffusive transport of soil gas, resulting in suboxic or anoxic conditions in soil microsites (Smith et al., 2003; Gregorich et al., 2014).

3.2.2 N Gas Emission as Function of Temperature

Like soil water content, temperature affects N_2O production and consumption both directly and indirectly. The direct effect of temperature is on the kinetics of enzyme activity (Billings and Tiemann, 2014), but temperature also indirectly affects N_2O production by influencing the number, type, and activity of nitrifying and denitrifying N_2O-producing microorganisms as well as the physiological processes performed by these organisms. Furthermore, enhanced microbial activity occurring as a result of higher temperatures leads to increased O_2 consumption during respiration creating microaerophilic or even anaerobic conditions that favor denitrification and increase N_2O production.

4. ROLE OF AGRICULTURAL MANAGEMENT PRACTICES

Every facet of agricultural management has an impact on soil physical, chemical, and biological properties and processes and thus can influence nitrogenous gas emissions from soils (Figure 6) and the resultant greenhouse gas effects. Addition of fertilizers and manure to supply N and organic matter for crop growth is one of the most important management practices that affect N emission because it supplies mineral N, the basic substrate for nitrification and denitrification processes. The type of inorganic or organic fertilizer, the rate at which it is applied, the time of application and where it is placed in or on the soil all affect the kinetics of production and emission of N gases. Tillage affects the soil structure in multiple ways (e.g., aggregation processes and pore space properties) and these structural changes in turn

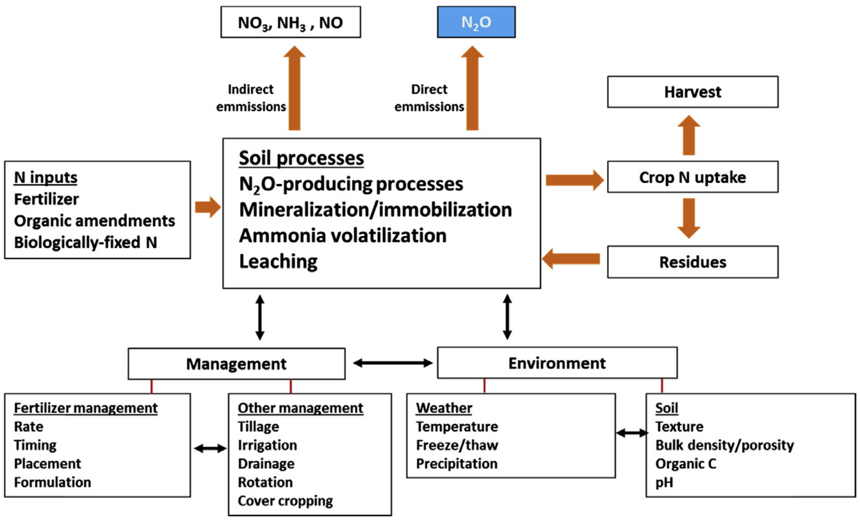

Figure 6 Factors influencing direct and indirect N_2O emissions from cropping systems. *Adapted from Venterea et al. (2012).*

affect the amount of oxygen and N gases and their diffusive transport through the profile to the soil surface. The type of crop grown, its sequence in the crop rotation, and the amount of crop residues returned to the soil are important because they affect the release and availability of available C and N (Aulakh et al., 2001; Baggs et al., 2003).

4.1 Synthetic Fertilizers

4.1.1 Synthetic Fertilizers—Rate and Type or Formulation

The rate and type of synthetic fertilizers both have an effect on N gas emissions from soil. Nitrous oxide emission is often related exponentially to N fertilizer application rate (Chantigny et al., 1998; Ma et al., 2010), but methodologies proposed by the IPCC (Smith et al., 2007) assume a linear response, typically amounting to 1−1.2% of the N applied (Bouwman, 1996; Helgason et al., 2005; Gregorich et al., 2005; Mosier et al., 2006). This loss rate is highly variable and can vary by an order of magnitude (Rochette et al., 2008c). Once the crop N needs have been met, the N_2O emission rates appear to increase nonlinearly (Malhi et al., 2006; McSwiney and Robertson, 2005; van Groenigen et al., 2010).

Anhydrous ammonia, urea, and urea ammonium nitrate (UAN) are the most common forms of N fertilizer used in North America (Ribaudo et al., 2011). A review of studies from around the world suggested that there is no

significant difference in N_2O emissions from soils receiving these forms of N fertilizers (Stehfest and Bouwman, 2006). However, side-by-side experiments in the United States and Canada suggest that the type of N fertilizer applied can sometimes have a significant effect on the magnitude of N gas emissions. In maize cropping systems, N_2O emissions were higher from soils receiving anhydrous ammonia than from those receiving UAN or urea (Thornton et al., 1996; Gagnon et al., 2011; Fujinuma et al., 2011). The difference in emissions between the two forms of N fertilizer may be due to the placement/location of the fertilizer: anhydrous ammonia is injected into the soil, whereas urea is broadcast. In a semiarid wheat cropping systems, however, no significant difference in N_2O emissions was found in a comparison of soils receiving either urea or anhydrous ammonia (Burton et al., 2008), perhaps reflecting the lower N application rate in wheat systems under dry climate.

Studies by Venterea et al. (2005, 2010) showed that tillage effects on N_2O emissions may depend on the form of N applied. They observed no difference in N_2O emissions between conventionally tilled and no-till systems when using UAN, but higher N_2O emissions from soil under no-till than from conventionally tilled soil when using broadcast urea. Emissions following anhydrous ammonia were higher in conventionally tilled than in no-till systems.

4.1.2 Synthetic Fertilizers—Time of Application

Proper timing of N application is probably the biggest challenge in managing N fertilizer: matching adequate levels of soil mineral N with the uptake of the added N by plants, without limiting their growth. Higher N_2O emissions (as well as N leaching losses) will occur when soil mineral N exceeds plant uptake. This synchrony is notoriously difficult in crops like maize where the crop demand for N is very low early in the growing season, then increasing rapidly during vegetative growth (i.e., V6-V7 stage), before dropping sharply as the crop matures. Most N fertilizers are applied early, either in fall or in spring, when there is no crop present or when the crop is small and N demand is low, (e.g., about one-third of the maize in the United States is fertilized in the fall (Paustian et al., 2004; Ribaudo et al., 2011)), which can lead to large losses of N.

Improved synchrony may be achieved with side-dress fertilizer applications, which are applied later in the growing season and "enhanced-efficiency fertilizers" (i.e., controlled-release N fertilizer; nitrification and urease inhibitors), and a combination of these two approaches.

4.1.3 Synthetic Fertilizers—Effects on Microorganisms

Long-term N fertilization can increase or decrease microbial abundance and population size of N_2O producers and consumers. Changes in abundance are primarily exerted by increasing the amount of plant-derived C inputs, by short-term toxicity of concentrated N or through changes in soil pH, which strongly interacts with N fertilizer to determine microbial abundance. In soils of neutral to high pH, N fertilizer has a positive effect on microbial abundance, while in soils with pH below 5, N application decreases microbial abundance (Geisseler and Scow, 2014). Since pH is also a strong determinant of community composition (Fierer and Jackson, 2006; Lauber et al., 2009), the acidifying effect of N fertilization may influence N_2O emissions by changing the relative abundance of different N_2O producers and consumers.

Bacterial (AOB) and archaeal (AOA) ammonia oxidizers are thought to have different pH optima (Nicol et al., 2008) with AOA dominating at low pH. In contrast, AOB abundance is positively correlated with increasing pH and these organisms may be absent in low pH environments (Prosser and Nicol, 2012). Some evidence suggests that ammonia sensitivity is quite varied within both AOB and AOA and may govern activity (Jia and Conrad, 2009; Di et al., 2010; Yao et al., 2013). However, the short- versus long-term impacts of N fertilization on ammonia oxidizer community composition and activity are not well understood. Further evidence is needed to unequivocally demonstrate the existence of different ecological niches for the two groups (Taylor et al., 2012; Prosser and Nicol, 2012).

Type of N fertilizer can also affect microbial abundance and community composition. Application of urea and ammonium salts leads to very high concentrations of N (NH_3 and NH_4^+) toxic to microorganisms (Omar and Ismail, 1999), but this appears to be short lived (Geisseler and Scow, 2014). Anhydrous ammonia fertilizer on the other hand appears to have long-term toxic effects compared to urea on soil microorganisms and may result in decreased microbial biomass, including nitrifiers (Biederbeck et al., 1996).

4.2 Crop Residues and Organic Amendments

Incorporation of crop residues affects the emission of N gases from soil because the N content of the residues controls mineralization/immobilization processes, thereby affecting the availability of N for nitrification/denitrification processes. The magnitude of the emissions depends on the quantity, quality, and timing of incorporation (Aulakh et al., 2001; Baggs et al.,

2003; Garcia-Ruiz and Baggs, 2007; Millar et al., 2004). Residues with relatively narrow C:N ratio have been shown to induce higher N_2O emissions than those in soils amended with residues having wide C:N (Toma and Hatano, 2007; Gomes et al., 2009). These residue quality effects may last for more than a single season; Liu et al. (2011) observed that incorporation of wheat straw increased N_2O and NO emissions in the following maize season, whereas incorporation of maize residues had no influence on emissions.

The effects of residues on substrate availability have been observed in studies examining the microbial response to residue removal. For example, Nemeth et al. (2014) found that removing crop residues increased N_2O emissions, which were correlated with lower copy number and transcription of *nos*Z, suggesting that the increased emissions were the result of incomplete reduction of N_2O to N_2, rather than to an overall increase in the amount of N denitrified. Furthermore, they showed that emissions in no-residue versus residue treatments were related to edaphic factors of C and N availability during de novo denitrification throughout a spring thaw event. Pelster et al. (2013) observed that return of crop residues decreased N_2O emissions by immobilizing N during freeze-thaw under controlled conditions. Hence there are several lines of evidence to suggest that the balance of N availability for denitrification with the timing of *nos*Z transcription is a critical factor in determining the N_2:N_2O ratio produced and that this balance is coupled to residue decomposition.

Application of manure to soil usually increases N_2O emissions through stimulation of nitrification and denitrification processes (e.g., Chang et al., 1998; Rochette et al., 2008a). Manure may contain large amounts of mineral and labile organic forms of N as well as large amounts of soluble organic C, an important driver of denitrification. Response to manure addition may therefore depend on the levels of available mineral N and soluble organic C in the soil and in the manure.

Chantigny et al. (2010) observed that manure application led to lower N_2O emissions in a clay soil, but higher emissions in a loam soil, when compared with N fertilizer application on the same soils. They attributed the higher emissions in the loam soil to the availability of organic C in the manure, which provided the substrate for denitrifying bacteria. Likewise the level of available mineral N may be important in determining the response to manure addition. Jarecki et al. (2009) observed that adding swine manure increased cumulative N_2O emissions but N availability was the primary controlling mechanism because N_2O emissions were not related to %WFPS.

Denitrification can also be induced as a result of increased oxygen consumption due to intense decomposition of manure shortly after its application (Smith et al., 2003). Thus relatively large emissions often occur within a short time after application of manure (Whalen, 2000); for example, Rochette et al. (2008a) reported that up to 60–90% of seasonal (i.e., May–November) N_2O emissions occurred within 40 days of manure application. But the manure effects on nitrification/denitrification processes can be long lived. Ellert and Janzen (2008) detected an increase in N_2O emissions over a 3-year period following a single manure application.

Application of biochar affects N gas emissions because it changes soil physical and chemical properties such as soil structure, water holding capacity, organic matter content, nutrient concentration, and pH (Lehmann and Joseph, 2009). It can affect microbial N cycling (and thus N gas production and emissions) through its effect on soil N concentration, mineral N absorption, and competition with plants for N uptake (Clough et al., 2013).

4.3 Tillage and Residue Management

The effect of tillage on N gas emission can be seen as a cascade of effects: tillage affects soil structure and aeration, soil temperature, and water content. All of these affect microbial activity, which in turn affects the rate of decomposition and N mineralization. However, the effects of tillage management practices on N gas emission is not consistent; some studies have shown decreased N_2O emissions when no-till or reduced tillage is implemented (e.g., Gregorich et al., 2007; Mosier et al., 2006), others reported higher emissions (e.g., Ball et al., 1999; Burford et al., 1981), while still others reported no difference (e.g., Lemke et al., 1999; Rochette et al., 2008b). Some research has shown that the effects of tillage on N_2O emissions depend on the type of tillage management implemented and on placement of N fertilizer (Drury et al., 2006; Venterea et al., 2005).

From a review of literature Six et al. (2004) concluded that soil N_2O emissions usually increase during the first 10 years after tilled soils have been converted to no-till; but this effect appeared to decrease over time and this reduction in N_2O emissions was apparent only in humid climates. van Kessel et al. (2013), in a meta-analysis of field studies (239 comparisons), showed that averaged across all comparisons, no-till/reduced till did not alter N_2O emissions compared with conventional tillage. However, no-till/reduced till significantly reduced N_2O emissions in experiments longer than 10 years, especially in dry climates. The effects of tillage on soil N_2O

emissions may be regulated to a large extent by climate. For example, in humid regions, like those in eastern Canada, soil N_2O emissions under no-till are more likely to be greater than those from conventional tillage, whereas in semiarid regions, like those in western Canada, the converse may be true (Helgason et al., 2005; Lemke and Janzen, 2007).

The gradually diminishing difference in N_2O emission between tilled and no-till systems may indicate the development of structure (Six et al., 2004); the formation of macropores and water-stable aggregates over time improves soil structure/aeration and decreases the formation of anaerobic microsites where N_2O is formed. Furthermore, the increase in microbial biomass observed under long-term NT (Helgason et al., 2009) may lead to an enhanced ability to immobilize N fertilizers at the time of application, resulting in a more gradual release throughout the growing season that is better synchronized with crop demand, particularly in dry climates. Smith et al. (2010) showed that tillage management affects the diversity of nitrifiers and denitrifiers and that these changes were apparent during peak N_2O emission events. However, a direct link between activity of these communities (i.e., gene transcription) and N_2O emissions was not assessed.

van Kessel et al. (2013) found no significant correlation between soil texture and the effect of no-till/reduced till on N_2O emissions. This is consistent with the findings of Skiba and Ball (2002) who concluded that the local influences of soil structure/soil water status interactions and soil mineral N content tend to override gross textural and drainage effects. These findings contrast with those of Rochette et al. (2008a), who, in summarizing 25 field studies, observed that no-till generally increased N_2O emissions in poorly aerated soils but had no effect in soils with good and medium aeration. They concluded that the impact of no-till on N_2O emissions is small in well-aerated soils but most often positive in soils where aeration is reduced by conditions or properties restricting drainage, such as is likely in no-till soils.

Microbial abundance is tightly linked to C availability and thus, to the quantity and quality of crop residues returned to the soil (Kallenbach and Grandy, 2011). Both residue type and placement affect microbial community composition (Nicolardot et al., 2007) and active decomposer communities have been shown to differ between surface-placed and incorporated residues (Helgason et al., 2014). Changes in residue decomposition under NT and CT can alter substrate availability (C and N) for nitrification and dentrification, affecting N_2O emissions.

4.4 Cropping Systems with Legumes

Symbiotic N fixation by legume crops contributes large amounts of N to agricultural soils, and the flow of this N through the plant—soil system can stimulate N_2O production from several processes. Cropping systems with legumes often produce lower annual N_2O emission than fertilized annual crops. The N_2O emissions from legume crops are derived mainly from decomposition of the above- and below-ground residues, and losses from the biological N fixation process per se are likely negligible (Rochette and Janzen, 2005).

Annual N_2O-N emissions in alfalfa cropping systems have been shown to be higher than those in soybean systems (Gregorich et al., 2005; Rochette et al., 2004). This may be the result of frequent cutting and harvesting of aboveground plant material on sources of N_2O in soil (Rochette et al., 2004), the litterfall of during the alfalfa growing season (Tomm et al., 1995); as well, biological N fixation may be higher in perennial alfalfa than in soybean. This is consistent with the observation that the dieback of alfalfa nodules occurs following harvest (Vance et al., 1979), which could contribute to N release from the root systems. Wagner-Riddle et al. (1997) measured high N_2O emissions in the spring following plow down of an alfalfa crop the previous autumn. This suggests that total N_2O emissions in alfalfa cropping systems may be greater than those only measured during the growing season. This highlights the importance of processes that contribute to the production of N_2O following harvest and plow down of N-rich crop residues.

4.5 Rotation

Rotation effects on N_2O emissions relate mainly to the amount of fertilizer N and the quantity and quality of crop residues returned to the soil. Meyer-Aurich et al. (2006) compared estimated N_2O emissions in six maize-based rotations under moldboard and chisel plow systems in a 20-year field experiment in eastern Ontario. Differences in emissions between tillage systems were small in comparison to rotation effects; crop rotations with legumes had substantially lower N_2O emissions than continuous maize, reflecting the higher inputs of fertilizer N in the monoculture system.

Maize—soybean rotations often have lower cumulative N_2O emissions compared to continuous maize production, because of lower seasonal N_2O emission from soybean than from maize (Rochette et al., 2004; Mosier et al., 2006; Hernandez-Ramierz et al., 2009). Carryover of N_2O emissions

from corn to soybean years within the rotation seems minimal (Grandy et al., 2006; Mosier et al., 2006; Parkin and Kaspar, 2006; Hernandez-Ramierz et al., 2009).

4.6 Irrigation and Drainage

Irrigation typically is assumed to increase direct N_2O emissions systems because water-filled pore space is expected to be greater than that in rain-fed systems. Studies to verify such assumptions, however, are scant because irrigation tends to confound comparisons. Since irrigated systems tend to be more productive, greater amounts of reactive N are circulating. Furthermore, irrigation water tends to be applied when transpiration rates are high, so periods when soils are moistened by irrigation often do not coincide with periods of high fluxes (Ellert and Janzen, 2008). Most studies do not directly compare the same crop with and without irrigation, but in a multi-year study of maize in Colorado, USA, soil N_2O emissions were smaller in a year with greater inputs of rainfall plus irrigation even though the latter culminated in greater proportions of water-filled soil pores (Halvorson and Del Grosso, 2013). In a recent review of soil N_2O emissions from irrigated and nonirrigated systems, Trost et al. (2013) concluded that the primary factor responsible for increased emissions from irrigated systems was usually increased amounts of available soil N, rather than increased water-filled soil pores. Since soil N_2O is a transitory intermediary, the balance between production and consumption is not expected to follow a straight-forward relationship with water-filled soil pores, as influenced by irrigation. Between 20% and 60% water-filled pore space, N_2O leakage during nitrification may predominate; from 60% to 75% water-filled pore space, N_2O from denitrification may predominate; but at greater than 75%, consumption by denitrification may even decrease soil N_2O fluxes. After accounting for the significantly increased yields with irrigation observed in many studies (e.g., Maharjan et al., 2014), N losses per unit of yield did not differ and in some cases decreased with irrigation.

5. AGRONOMIC ASSESSMENT OF N GAS EMISSIONS AND BROADER ENVIRONMENTAL CONTEXT OF N FERTILIZERS

5.1 Yield-Scaled Emissions

To satisfy the increased global demand for agricultural products, it will be necessary to strike a judicious balance between an agronomic perspective

of maintaining adequate N inputs and an environmental perspective of minimizing N losses from soil to the atmosphere. This concept is embodied in the approach where N_2O emissions are expressed as a function of crop productivity measurements like yield or N uptake (van Groenigen et al., 2010; Venterea et al., 2011). Thus N_2O emissions are thought of as a "cost of production," and the aim is to maximize returns per unit "cost." Scaling or normalizing the emissions to the quantity of crop produced helps put the emissions "cost" into perspective and allows more holistic comparison of management systems.

The yield-scaled N_2O emission metric is helpful to evaluate the effects of soil degradation on N_2O emissions because it captures both the negative environmental and agronomic aspects of soil compaction in a single parameter. Gregorich et al. (2014) observed that yield-scaled N_2O emissions were particularly sensitive to compaction because compaction both increased the area-scaled N_2O emissions (metric numerator) and decreased the yield (metric denominator) providing a double effect on the yield-scaled N_2O emission metric. Consequently, the ratios in yield-scaled N_2O emissions between compacted and not compacted soils were higher than the ratios in area-scaled N_2O emissions in the same year. In contrast, increasing N fertilizer increases both area-scaled N_2O emissions and yields. As a result, the ratio of yield-scaled N_2O emissions between high and low N fertilizer rates were less than the ratio of area-scaled N_2O emissions for high and low N fertilizer rates in the same year.

5.2 Fertilizer Use and Efficiency in Developing versus Developed Countries

Since 1960, N fertilizer use has steadily increased in all countries but the rate of increase has slowed in developed countries since about 1980, while in developing countries it has increased during this period (Figure 7; FAO, 2011). Recovery of N fertilizers by grain (maize, rice, and wheat) crops in field research plots can be as high as 85% but is generally 30–40% (Krupnik et al., 2004). Across regions of the world the average recovery for grain crops is lowest in Africa (26%) and this low efficiency use can be attributed to other limiting factors such as water and phosphorus deficiency (Krupnik et al., 2004). Across agricultural production systems the current fertilizer-N use efficiency is estimated to be about 50% (Smil, 1999; Ladha et al., 2005).

These losses of more than 50% of the applied fertilizer are dissipated across the wider environment by leaching of soluble N and emission of N gases to the atmosphere. The losses also represent significant economic

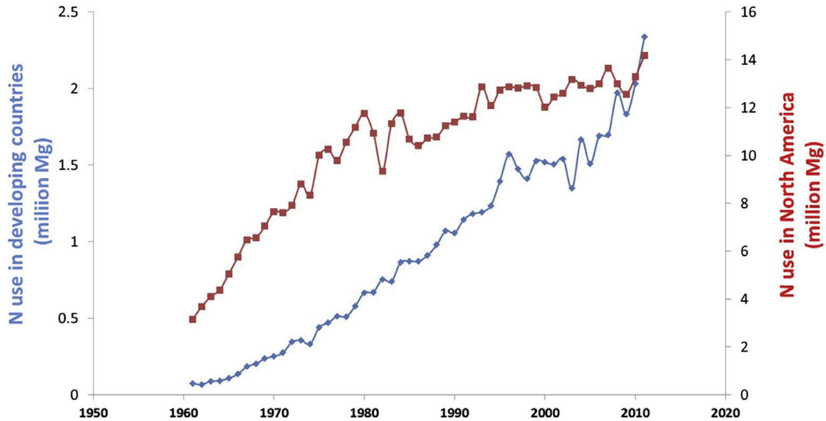

Figure 7 Trends of fertilizer N use in North America and developing countries from 1960 to 2010. *Data obtained from FAO (2011).*

loss to farmers and are particularly acute to small landowners in developing countries of Africa where fertilizer costs up to 5–7 times more than in developed countries (Sanchez, 2002).

Developing countries have been motivated to improve nitrogen use efficiency by economic factors. For example, starting in 2008 deep placement of urea was adopted on a wide scale in Bangladesh (IFDC, 2012) in response to the desire of the government to reduce national subsidies of fertilizer after marked increased in the price of fertilizer worldwide.

6. MITIGATION STRATEGIES

6.1 Management Options for Reducing N Gas Emission from Soil

Because of the prominence of N in agriculture systems, practices involving N management are critical in greenhouse gas mitigation strategies. Changing the application rate, source, placement, and timing of N fertilizer application have been suggested as ways, separately or in combination, to reduce soil N gaseous emissions in agricultural systems (e.g., Table 1).

While these management options can often reduce N gas emissions, economic barriers may limit their use. Farmers' profits are tied to yield, and therefore the use and success of these management options will depend on yield response, both spatially across the field and temporally over multiple growing seasons.

Table 1 Estimates of N_2O emissions reductions for a 15% reduction in N fertilizer rate

Region and crop type	Comments or caveats	Potential (t $CO_2e\ ha^{-1}\ yr^{-1}$)	Reference
USA national estimate	Estimated reduction of 30–40% with efficient use of N inputs		Paustian et al. (2004)
Global	Model from field estimates (n = 840); reduce N_2O by 8.2% at rates of 75–225 kg N ha^{-1}		Stehfest and Bouwman (2006)
Global	Reduce N application by 20%, dry versus moist climate, wide range in potential	Dry: 0.33 Moist: 0.62	Smith et al. (2007)
USA: Michigan, continuous corn; corn belt and Lake States, corn–soybean rotation	Calculated from field trials, assumes 15% reduction of N application	CC: 0.70 CS: 0.60	Millar et al. (2010)
USA: Kansas	Reduce application of urea fertilizer	0.02	Bremer (2006)*
USA: Colorado	Lower potential for continuous corn and for corn–barley rotation than for corn–dry bean rotation	0.08	Halvorson et al. (2008)*
USA: Colorado	Conventional tillage and no-till systems; continuous corn or corn–dry bean rotation	CT/CC: 0.06 NT/CC: 0.05 NT/CB: 0.04	Mosier et al. (2006)
USA: Michigan	2–7% of each additional kg N lost as N_2O, no yield decrease as long as rate remained above 101 kg N ha^{-1}	0.31	McSwiney and Robertson (2005)*

* In these studies, the potential mitigation is based on the relationship between N_2O and a given N fertilizer rate and then calculated under the assumption of a 15% rate reduction.

Adapted from Eagle et al. (2012).

Venterea et al. (2012) argue that a dual economic and environmental approach may be the best strategy for reducing N gas emissions. This is because focusing on a single factor, like fine-tuning the N rate to meet crop uptake, is inherently risky because crop yields and NO_3-N leaching losses depend on variations in annual weather. Rather than using a single option, a suite of management practices (e.g., improved fertilizer timing, placement, or source) could be applied, allowing for reduced N-rates while maintaining (or even increasing) yields without further financial incentives.

Furthermore, simply reducing fertilizer N inputs uniformly across a field(s) may not be a sound approach because of the high spatial variation in soil mineral N, mineralization processes and potential for N loss across a particular field. Therefore, site-specific fertilization designed to match within field variation in crop N requirements might allow for overall N-rate reductions without reducing yields. Sehy et al. (2003), for example, observed that in low-yielding areas application of precision-based N input resulted in one-third less N_2O released over a year following fertilization, while crop yield was not affected. But such spatially differentiated N-fertilizer applications may be complex because other factors besides soil nitrate contents, such as the supply of water and lateral nutrient, and annual variations in climate, especially rainfall, need to also be considered.

6.2 Improved Manure Management

Land application of animal manure increases N_2O emission and opportunities for reducing N losses from manure are possible because of inefficiencies in their use (Mosier et al., 1998; Petersen, 1999). Applying current management practices and technologies could potentially reduce N loss from farm and livestock operations to the environment by 30–50% (Robertson and Vitousek, 2009; Davidson et al., 2012). Such improvements are challenging, however, because manure is highly heterogeneous, including a range of N forms from those immediately available (e.g., soluble ammonium) to those which are only slowly released by subsequent decay (various organic N forms). Moreover, timing of application is more constrained for manure than for fertilizer; for example, in-crop application of manure is often not possible.

Globally, the amount of N in manure equals or exceeds that applied in fertilizer (Bouwman et al., 2013). Given the propensity of this N for loss, and the difficulties in synchronizing rates and timing with crop demands, wise use of manure N remains one of the most pressing opportunities for reducing gaseous losses of N (Petersen et al., 2013).

Long-term studies with manure show long and lingering effects of manure on subsequent N_2O emissions (Ellert and Janzen, 2008; Chang et al., 1998). Therefore focusing on the handling and application of manure, as well as how the soil is managed after application, will be critical features to consider in developing improved manure management strategies.

6.3 Enhanced Efficiency and Slow-Release Fertilizers

Enhanced efficiency and slow-release fertilizers have been developed to increase the efficiency of N fertilizer use by crops and decrease N losses by leaching and denitrification. Enhanced efficiency fertilizers are defined as those containing nitrification and urease inhibitors, and slow-release fertilizers are defined as those which have a coating or a modification, which slows the rate of N release. Nitrification inhibitors are compounds that delay bacterial oxidation of NH_4^+ by depressing the activities of nitrifier microorganisms in soil, whereas urease inhibitors are compounds that delay the hydrolysis of urea.

These enhanced efficiency and slow-release fertilizers could conceivably reduce the loss of N (by slowing nitrification) and thus indirectly reduce N gas emissions from soil (Freney, 1997; Wolt, 2004; Delagado and Mosier, 1996; Halvorson et al., 2010). Studies have shown that application of nitrification inhibitors significantly increased soil inorganic N and shifted the main soil inorganic N form from NO_3^- to NH_4^+ while concomitantly increasing crop yield, aboveground biomass and N uptake by aboveground plant (Liu et al., 2013).

Halvorson et al. (2008) observed that the spikes in N_2O emission were greater with UAN and urea than with polymer-coated urea. Other studies by Halvorson et al. (2010) showed that four enhanced-efficiency fertilizers reduced N_2O emissions (by 27–53%) in direct comparison to urea and UAN fertilizers. The N_2O-N loss, as a percent of N applied, was 0.3% for urea, but lower from all other N sources; grain production was not reduced by the use of the enhanced efficiency fertilizers. More recently Halvorson et al. (2014) measured reductions in N_2O emissions by about 40–60% for various enhanced efficiency fertilizers. In relatively humid areas, the effectiveness of enhanced efficiency fertilizer may be constrained by soil temperature and water content changes occurring over the growing season (Parkin and Hatfield, 2014).

A *meta*-analysis of field experiment data (113 datasets from 35 studies worldwide) by Akiyama et al. (2010) showed that nitrification inhibitors reduced N_2O emissions by about 38% and NO emissions by about 46%

compared with those from conventional fertilizers. A drawback of nitrification inhibitors is that they are often applied separately from fertilizers, thus increasing labor and fuel costs. Polymer-coated fertilizers also significantly reduced N_2O emissions by about 35% and NO emissions by 40% but urease inhibitors were not effective. The advantage of these fertilizers over nitrification inhibitors is that they reduce the requirement for multiple field operations thereby reducing labor and fuel costs (Grant, 2005). The effectiveness of nitrification inhibitors was relatively consistent across different types of inhibitors and soils and the inhibitors were effective in reducing N_2O emission from both chemical and organic fertilizers. Hatfield and Venterea (2014), reviewing field studies across the United States, concluded that enhanced efficiency fertilizers can decrease the N_2O emission, especially during the period immediately after fertilizer application and more consistently under irrigated production systems.

6.4 Land Sparing versus Land Sharing Debate

Globally the amount of arable land has not appreciably increased over the last 30 years, but over the same period the population has continued to grow; and it will likely continue to increase for about the next 30–40 years. With more and more people to feed on finite arable land, a debate has emerged about two competing approaches: land sharing (integration) versus land sparing (separation). This debate embodies concerns about biodiversity, food production, and security, and land scarcity (Fischer et al., 2014). Under a land-sparing strategy, some land is set aside and preserved for conservation and nonagricultural use while other land is intensively used to produce agricultural products; under a land-sharing strategy, less land is set aside specifically for conservation, but less-intensive production techniques are used on the agricultural land (Green et al., 2005).

To generate clear insights into the practical outcomes from this debate a discussion is needed to determine exactly what is to be conserved (e.g., a particular species or ecosystem service like air or water quality). This issue becomes relevant in the context of evaluating and selecting agricultural practices that involve management of N. In selecting a specific practice, there may be a debate as to whether it is preferable from an environmental perspective to use a practice with high ecosystem services (e.g., high soil C storage, low N_2O emissions)—a land-sharing strategy; or to use a practice using less land having lower ecosystem services but insuring high yields—a land-sparing strategy. A more direct assessment for the farmer would be to evaluate whether to use high rates of N fertilizer in

small areas or use legume-based systems in larger areas. Comparison of these strategies will inevitably be linked to crop yields because of the need to increase production for a growing global population and because economic considerations will be given for the farmers who need to make a profit. Thus in terms of considering N in the context of greenhouse gas effects there would be a need for an evaluation based on gaseous emissions per unit of agriculture commodity rather than on a per unit area basis.

6.5 New Opportunities

Meeting burgeoning food demands while minimizing gaseous and other leaks of reactive N into the environment has become a prominent global challenge for the coming decades (Sutton et al., 2011; Rockström et al., 2009). Resolving this objective will require intensive research efforts on several fronts.

6.5.1 Biological Developments

The suite of new genetic techniques now available may allow further improvements in the stemming of nitrogen leaks. Such enhancements may arise from an expanded understanding of the processes whereby microbial populations shuffles N atoms through the soil—plant system (e.g., Wei et al., 2014; Nemeth et al., 2014) leading eventually to management practices that discourage losses of N_2O or other N gases (Butterbach-Bahl et al., 2013). Or it may be possible to select for or develop crop genotypes with higher N use efficiency, allowing farmers to maintain yields with reduced N inputs (e.g., Zebarth et al., 2009; Bi et al., 2014; Xu et al., 2014). Another approach may be to use gene expression profiling to monitor N status of the growing crop (Zebarth et al., 2011).

Richardson et al. (2009) summarized the potential for mitigating N_2O release by manipulating enzymes involved in dentrification with a particular focus on enhancing the conversion of N_2O to N_2. One approach could be to use plant breeding to manipulate denitrification through inputs into the plant rhizosphere, thereby changing the composition of plant-derived C flow or N uptake demand. Furthermore, enzyme activities might be controlled by manipulating the availability of required metal cofactors such as Cu, Fe or Mo—and thereby ultimately causing a reduction in N_2O emissions. Another possibility is that plants could be genetically engineered to express N_2O-reductase. Such approaches, however, will demand broad and long-term ecosystem evaluation. Moreover, because N_2O fluxes

are often highest between growing seasons, the effectiveness of plant-based techniques may sometimes be limited.

6.5.2 Technological Developments

A fundamental approach to suppressing N_2O and other gaseous losses is to synchronize available N precisely with plant uptake throughout the year. This aim may be advanced in coming years by a range of emerging technologies, including the development of improved fertilizer forms or amendments (e.g., Azeem et al., 2014; Halverson et al., 2014; Decock, 2014) and the use of new sensors to more accurately predict short-term plant demands (e.g., Raper et al., 2013; Muñoz-Huerta et al., 2013; Xue et al., 2014).

6.5.3 Social Developments

Avoiding gaseous losses depends not only on technological and biological breakthroughs, but also on the widespread adoption of these improved practices or policies. This depends on effective two-way communication between researchers and farmers, allowing appropriate practices to be tuned to local agroecosystems (Davidson et al., in press). As well, it may require innovative policies and incentives, which encourage their adoption, especially where the practices or approaches may involve some short-term economic costs. Although researchers traditionally focus on developments in technology and scientific understanding, the human element may be no less important.

No single development, enacted alone, is likely to resolve the challenge of reducing gaseous N emissions in a hungrier world. Indeed, progress is likely to be favored most by a collective effort, forging a broad coalition of disciplines to study farms as food-producing ecosystems, of which humans are an integral part, both as practitioners and beneficiaries.

Sutton et al. (2014) argue that the "Sector view" (green actions consistent with improved profit) and "Societal view" (incorporating the value of all externalities) need to be brought together to view the control of N_2O emissions with a long-term perspective while accounting for all cobenefits of taking action. In this way N_2O control is viewed within the context of the wider N cycle with an emphasis on improving full-chain N use efficiency by exploiting a combination of technical measures and management practices (Figure 8). Considering N use efficiency in the context of the N cycle links the control of N_2O emissions with other N losses (e.g., NO_x, NH_3, and NO_3^-) and thus contributes to mitigation of N pollution in the environment (Reay et al., 2012).

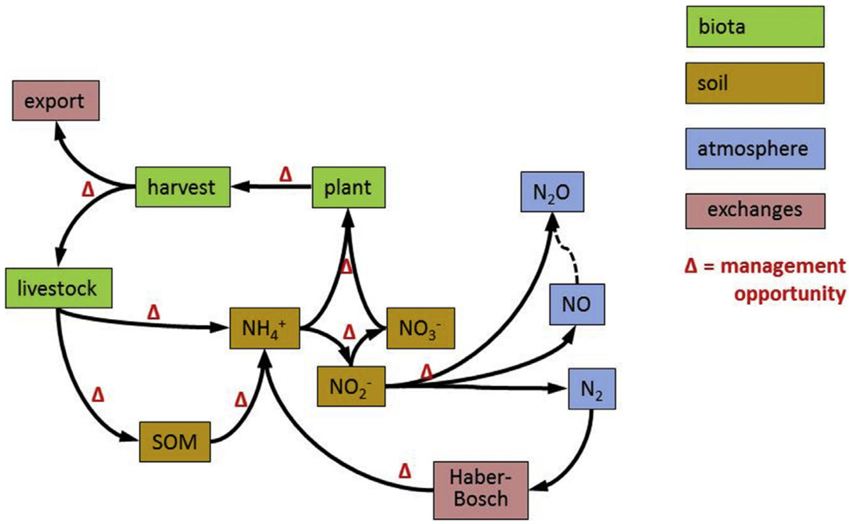

Figure 8 Flows of N among the atmosphere, soil and biota (including plants and animals) showing the points at which management opportunities exist to affect the flows.

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CHAPTER THREE

Hydrological Aspects of Arsenic Contamination of Groundwater in Eastern India

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Contents

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Abstract

Arsenic in the Bengal Delta has been the topic of discussion for more than a decade since 73% of the population (more than 140 million people) are under deep stress for locating arsenic-free drinking water. Most of this arsenic is geogenic, having its source from the Himalayan metamorphic facies, and is carried down by major river systems and thereby accumulating on the fluvio-deltaic plains to the southeast. All the states on this path are to some extent have depicted the presence of arsenic in the subsurface groundwaters. Currently, there are two major pathways for ingesting arsenic: (1) by the drinking water; and (2) by the food chain. Various scales of health effects from skin lesions to major cancer outbreaks have been located distinctively and pathologically in about 43% of the affected population. Irrigation practices have exacerbated the lateral extent of high arsenic in this region. Secondary natural minerals Fe and Mn oxyhydroxides seem to adsorb both the valences of arsenic and, in the presence of labile organic carbon as in dissolved organic matter, help in releasing the arsenic into the groundwaters. Shallow to intermediate depth-reducing aquifers within the Holocene floodplains are the most contaminated and range from <10 to 4600 $\mu g\ L^{-1}$. The Bengal Basin, within a few meters of sea level, has repercussions from infiltrating saline seawater, and the impacts of climate change makes the problem worse in this region. Switching groundwater wells, harvesting rainwater, and household small-scale filtration systems, are the three most effective remediation mechanisms known to the people of the Bengal Delta.

1. INTRODUCTION

Arsenic is a common element found throughout the atmosphere, hydrosphere, geosphere, and biosphere. It has been identified as toxic to humans and maximum contamination levels have been established by local national governments and international regulatory organizations. Based on ecotoxicological studies, the drinking water standard in Bangladesh and West Bengal is 50 $\mu g\ L^{-1}$, while the United States Environmental Protection Agency and the World Health Organization have established their maximum contaminant level (MCL) at 10 $\mu g\ L^{-1}$. As contamination of groundwater poses a serious health threat to millions of people across the globe. The As problem is accentuated in southeast Asia where access to clean sanitation and drinking water sources are a challenge. Groundwaters in the Bengal Basin, located in eastern India and Bangladesh, have been found to

contain elevated concentrations of As, which affect tens of millions of inhabitants. This has been described as the worst global environmental calamity (Smith et al., 2000) and extensive research initiatives have been developed to address this problem for the past decade and more.

In the 1970s, millions of people in the Bengal Basin were utilizing surface water infected with pathogens for drinking purposes. This lead to extensive diarrheal diseases and mortality among people. In response to this health crisis, UNICEF intervened and drilled thousands of 3-inch tube wells into a shallow aquifer to provide safe drinking water to residents (Smith et al., 2000). As concentrations in the groundwater were not initially measured and in 1978 the first concentration $>50\ \mu g\ L^{-1}$ was detected in West Bengal (Saha, 1995; Acharyya et al., 2000). In 1983 the first case of human As poisoning was identified in West Bengal and by 1987 cases from Bangladesh began to be identified (Das et al., 1994; Chatterjee et al., 1995; Dhar et al., 1997; Smith et al., 2000). An extensive survey of As contamination in West Bengal was launched that lasted 12 years and concluded that between 3 and 4 million people across 6 districts of West Bengal were affected by elevated concentrations of As (Acharyya et al., 2000; Smith et al., 2000). The Department of Public Health and Engineering (DPHE) in Bangladesh first identified high As concentrations in Bangladesh groundwater in 1993 (Acharyya et al., 2000; BGS/DPHE/MML, 2001). In 1995, the School of Environmental Studies at Jadavpur University in Calcutta hosted a conference addressing the As contamination issue and brought the problem under international attention (Chakraborti et al., 2013; Acharyya et al., 2000; BGS/DPHE/MML, 2001). Following this conference, extensive surveys by several organizations were conducted throughout the region to identify the magnitude of the problem. The British Geological Survey (BGS) report on As contamination in Bangladesh contains details of their survey and overviews of the UNICEF and DPHE programs. Table 1 displays the extent of As contamination in various regions of West Bengal (Mukherjee et al., 2005).

There are several ways As can contaminate subsurface groundwater and can generally be classified as natural or anthropogenic. Geogenic sources of As derive from volcanic activity, thermal springs, and weathering reactions (Bhattacharyya et al., 1997; Chakraborti et al., 1998; Smedley and Kinniburgh, 2002). Anthropogenic sources of As include application of pesticides, mining, landfill leaching, and discharge of industrial effluent (Bhattacharyya et al., 1997; Chakraborti et al., 1998). There have been some cases of anthropogenic As contamination in Calcutta and throughout India (Chakraborti

Table 1 As concentrations in selected regions of West Bengal

Location	Area affected (km^2)	Depth of well (m)	As concentration ($\mu g\ L^{-1}$)
West Bengal	34000[a]	14–132[a]	<1–1300[a]
Murshidabad district			
Beldanga Block			
Hand tube well		35–45[b]	<10–4622[b]
Hariharpara Block			
Hand tube well		6–37[b]	5–695[b]
Jalangi Block			
Hand tube well		20.12–64.02[c]	133 (21–176)[c]
Shallow tube well on agricultural land		14.02–20.12[c]	108 (40–182)[c]
Domkal Block			
Hand tube well		7.93–73.17[c]	100 (6–138)[c]
Shallow tube well on agricultural land		14.02–26.61[c]	85 (18–200)[c]
Bhaga-bangola-1 Block			
Hand tube well		–	<10–150[g]
Bhaga-bangola-2 Block			
Hand tube well		–	<10–100[g]
Nadia district			
Chakdaha Block			
Hand tube well		5–126[d]	112 (1–267)[d]; 221 (1.1–476)[e]; 260 (68–452)[f]
24-Parganas (South) district			
Baruipur Block			
Hand tube well		14–305[d]	283 (1–1059)[d]; 351 (9–693)[f]

Adopted from Mukherjee et al. (2005); Data from (a) Bhattacharya et al. (2002a); (b) Sankar et al. (2014); (c) Roychowdhury et al. (2003); (d) Bhattacharya et al. (2001); (e) Nath et al. (2011); (f) Bhowmick et al. (2013); (g) Samadder (2010). Reprinted from Murkherjee et al., 2005, with permission from CSIRO Publishing http://www.publish.csiro.au/pid/3478.htm.

et al., 1998). One plant in particular was examined by Chakraborti et al. (1998), and it was found to produce an As-based insecticide called "paris green." Unregulated disposal of industrial effluents had contaminated local soil and groundwater and adversely affected residents. The plant was shut down and mitigation measures were taken to reduce future health impacts (Chakraborti et al., 1998). The As problem in the Bengal Basin is not a result

of these type of human activities as there are no mines or extensive industrial activity in the basin (Acharyya et al., 2000). Natural contamination of groundwater is suspected to be the cause of As enrichment in eastern India and all through the Bengal Basin (Bhattacharya et al., 1997; Acharyya et al., 2000). Natural As contamination of groundwater is a complicated mechanism dependent on local geology, hydrology, geochemistry, and anthropogenic activities collectively (Bhattacharya et al., 1997).

In light of the massive contamination of the Bengal Basin, extensive research has been performed examining the mechanisms affecting As release and mobilization, the influence of local geology and hydrology and the impacts of agricultural irrigation practices on As concentrations.

2. HEALTH EFFECTS

Health effects caused by long-term exposure to high As concentrations are serious and manifest in a variety of diseases. Studies from Bangladesh indicate that 60% of the total daily intake of As comes from drinking water and the remaining from crops grown using irrigation (Naidu et al., 2005). As skin lesions were first detected in West Bengal in 1983, which set off a huge survey of As contamination throughout India and Bangladesh (Saha, 1995). Clinical studies have revealed that an As concentration at or above 300 $\mu g\ L^{-1}$ can lead to severe As-related disease (Chowdhury et al., 2000). Bodily organs most affected by As exposure include the circulatory system, liver, kidney, gastrointestinal tract, and skin (Duker et al., 2005). Clinical studies from West Bengal have found the following As-related diseases: diffuse melanoma, spotted melanosis, leucomelanosis, mucus membrane melanosis, diffuse keratosis, spotted keratosis, hyperkeratosis, gangrene, squamous cell carcinoma, and various cancers (skin, liver, kidney, lung, and bladder) (Anawar et al., 2002, and references therein). Melanosis is the darkening of the skin and keratosis is the roughening and hardening of the skin due to long-term exposure of low to medium As concentrations. Long-term exposure to high As levels can lead to carcinoma, which can include skin cancer and liver and spleen damage (Mandal et al., 1996). A comprehensive overview of the health effects of As exposure is beyond the scope of this paper and readers are referred to other specialized literature e.g., Check for the paper by Kapaj et al. (2006). Duker et al. (2005) gives an overview of As geochemistry and health effects. Mandal et al. (1996) describes the development and manifestation of As poisoning in residents of

West Bengal, India. Anawar et al. (2002) gives an overview of the health effects found throughout Bangladesh. Das et al. (2012) examine the increased risk of liver and cardiovascular disease due to As exposure in populations within West Bengal, India. Rahman and Al-Muyeed (2005) survey the health of residents of the severely affected Rajipur village in West Bengal. Effects of As in the environment and the potential impact on human health through various pathways need to be studied in detail.

Groundwater arsenic contamination in Ganga–Meghna–Brahmaputra plain, its health effects and an approach for mitigation, Dipankar Chakraborti, Figure 1.

3. EXTENT OF THE PROBLEM

It is estimated that between 70 and 100 million people are affected by As contamination in India and Bangladesh (Ng et al., 2003). An estimated 90% of drinking water in India and Bangladesh are derived from shallow (<300 m) groundwater wells and over one million wells are suspected to have As concentrations >50 $\mu g\ L^{-1}$ (McArthur et al., 2001). Figure 1 depicts the regions affected by and at risk of As contamination in the Bengal Basin. In India, eight states have been identified as affected by As-enriched groundwater: West Bengal, Uttar Pradesh, Bihar, Jharkhand, Assam, Chhattisgarh, and Madhya Pradesh. Within these states, districts have been classified as severely affected (As >300 $\mu g\ L^{-1}$), mildly affected (As >50 $\mu g\ L^{-1}$), and not affected (As <10 $\mu g\ L^{-1}$) by the School of Environmental Studies, Jadavpur University. A recent overview of the As contamination of India by Nickson et al. (2007) describes the methods employed to determine the extent of the problem in West Bengal, Bihar, Uttar Pradesh, Assam, and Jharkhand.

In 1974, India built a barrage to divert the water on the Ganges River at Farakka to the Hoogly River. Since the consensus is that the source of As is geogenic, the river diversion has not been attributed as a source of As. However, recent studies raise the question that the diversion created deficiency of water downstream and resulted in increased As in the region (Chatterji et al., 2002).

3.1 West Bengal

West Bengal is located in eastern India and is bordered by Bangladesh to the east. 80.2 million people live in the 19 districts of West Bengal over an area

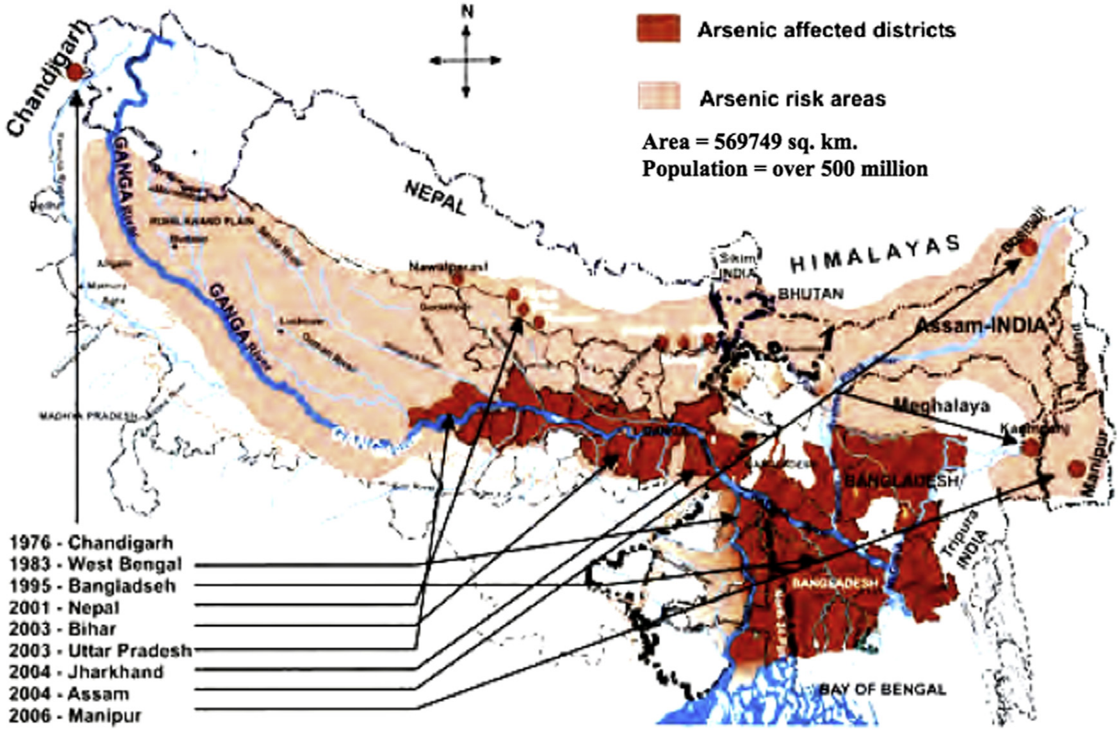

Figure 1 Map depicting the As affected districts and risk areas in Ganga–Meghna–Brahmaputra Plain. *Adapted from Chakraborti et al. (2008). With kind permission from Springer Science and Business Media, Environ. Earth Sci. 70 (5), 2013, 1993–2008.*

of 88,750 km^2. As contamination in India and Bangladesh was first discovered in West Bengal and it is arguably the most affected state in India. Extensive studies throughout the 19 districts have provided a solid overview of the extent of As contamination. The School of Environmental Studies (SOES) has analyzed 140,150 tube well samples from all 19 districts. From these findings they determined that 48.1% exceed 10 $\mu g\ L^{-1}$, 23.8% exceed 50 $\mu g\ L^{-1}$, 3.3% exceed 300 $\mu g\ L^{-1}$, and 0.13% are above 1000 $\mu g\ L^{-1}$. This puts 50.4 million people in 37,910 km^2 at severe risk over an area of 38,861 km^2 (SOES, 2006). Nine districts are severely affected (Nadia, Murshidibad, Malda, Burdwan, Hoogly, Howrah, and Kolkata), five are mildly affected (Kochbihar, Jalpaiguri, Darjeeling, North Dinajpur, and South Dinajpur) and five are not affected (Bankura, Birbhum, Purulia, Medinipur East, and Medinipur West) (SOES, 2006; Bhattacharya et al., 1997). Figure 2 exhibits a map highlighting the contaminated blocks within West Bengal (Nickson et al., 2007). We will discuss the extent of contamination in the most severely affected districts in West Bengal, India.

3.1.1 Nadia

Nadia covers an area of 3927 km^2 with a population of 4,604,827. It is divided into 17 blocks with 1250 villages on record. Nadia hosts some of the highest occurrences of As in drinking water in the Bengal Delta. The Chakdaha block of Nadia, bounded on the west by the Hooghly River and east by the Ichamati River, has been extensively studied (Nath et al., 2008). The general slope of the area is southeast with small rivers, streams, and swamps (Nath et al., 2005). Results show groundwater in the Nadia district has mostly Ca-HCO_3 facies (Mukherjee et al., 2008). The depth distribution for the Nadia district area coincides with other research that As concentration in groundwater generally decreases with increasing depth. The SOES has collected 28,794 tube well samples from Nadia and found that 51.2% > 10 $\mu g\ L^{-1}$, 17.2% > 50 $\mu g\ L^{-1}$, 1.8% > 300 $\mu g\ L^{-1}$. 13 samples exceeded 1000 $\mu g\ L^{-1}$ and the maximum concentration was 3200 $\mu g\ L^{-1}$ (SOES, 2006).

Samples collected from wells in the Chakdah area in Nadia show As concentrations exceeding 50 $\mu g\ L^{-1}$ in close proximity to low As concentrations (Pal et al., 2002). Elevated As concentrations have been found in rice from Nadia and presents an additional exposure route for As contamination (Mondal et al., 2010). In the Chakdha block, 2% of the surveyed population was found to be using drinking water from As removal treatment plants (median As = 74 lg L^{-1}), 28% from deep tube wells (median As = 34 lg L^{-1})

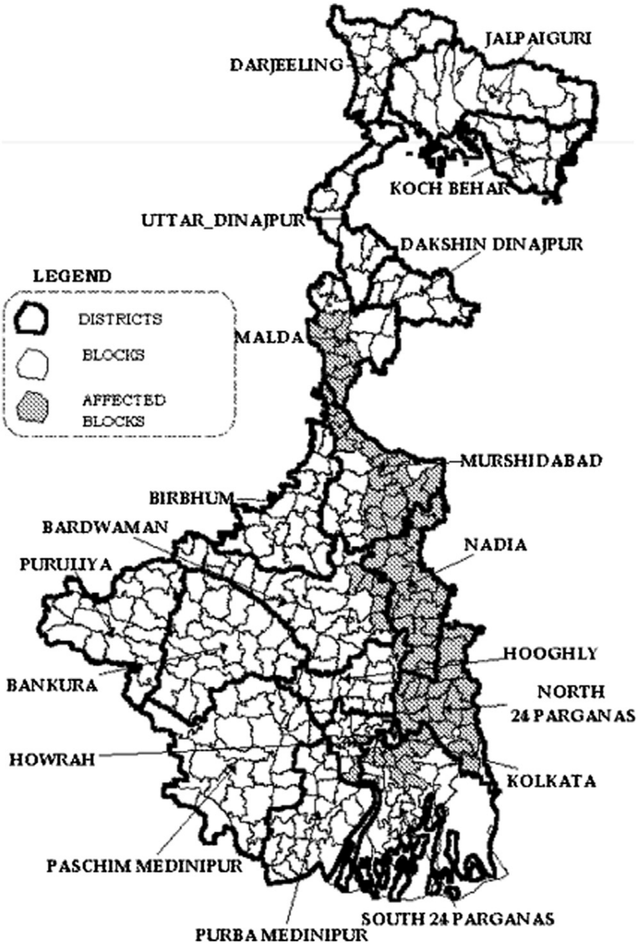

Figure 2 Map of As contaminated blocks in West Bengal, India. *Adapted from Nickson et al. (2007). Reprinted by permission of the publisher Taylor & Francis Ltd, http://www.tandfonline.com.*

and 15% from tap water (median As = 20 lg L^{-1}) while the remaining wells were private tube wells (Mondal et al., 2010).

Between 1995 and 2000, the Nadia district began increasing the number of shallow tube wells compared with deep tube wells. It was noted that the Nadia administration is not encouraging installing new irrigation systems that extract groundwater (Nath et al., 2008). Nath et al. (2008) found 88% of the investigated wells to have concentrations higher than the WHO guideline value. High concentrations were found in two hot spots, one at the Chakdaha city center and the other on the western edge of the

city. The hot spots are areas containing high As (>200 μg L^{-1}) and occur within 100 m to those with low As (<50 μg L^{-1}) (Nath et al., 2008).

Chronic As toxicity symptoms in West Bengal and Bangladesh may reflect pathways other than consuming water (Mondal and Polya, 2008). Exposure from As from consumption of foods has been studied in the Chakdaha region of Nadia. Efforts are currently focusing on reducing As from drinking water, with less emphasis on As exposure from soil to crop transfer or cooking in As contaminated water. As rich water is used extensively during the dry season for crop irrigation. Mondal and Polya (2008) studied As concentrations in rice in the Chakdaha block and found a median lifetime cancer risk from cooked rice of 7.62×10^{-4}, which is higher than the 10^{-4}–10^{-6} range typically used by the USEPA as a threshold for regulatory values. Median As exposure from cooked rice was found to be 0.50 μg kg^{-1} d^{-1} for the Chakdaha block (Mondal et al., 2010). Five blocks of the Nadia district investigated As contamination in rice, wheat, and vegetables with total As concentrations varying between <0.0003 and 1.02 mg kg^{-1} (Bhattacharya et al., 2010). Potato had the highest mean As concentrations with 0.654 mg kg^{-1}.

3.1.2 Murshidabad

Murshidabad is bordered by the Ganga River to the East and North which forms the border with Bangladesh. It covers an area of 5324 km^2 and has a population of 5,866,569. Twenty six blocks contain 1918 official villages in Murshidabad. Analyses of 29,668 tube well samples reveal that 53.8% > 10 μg L^{-1}, 26.7% > 50 μg L^{-1}, 4.4% > 300 μg L^{-1} 71 samples exceeded 1000 μg L^{-1} and the maximum concentration was 3003 μg L^{-1}.

Levels of As east of the Bhagirathi were found to be as high as >4000 μg L^{-1} (Neal et al., 2010). The high-valued hot spots of As east of the Bhagirathi tend to be localized, occurring primarily along the Holocene floodplains. Lower values of As are found west of the Bhagirathi River and also to the west of the Ganges–Brahmaputra and Meghna Rivers.

The Bhawangola-I block of the Murshidabad district obtains 10% of its water from deep tube wells (median As = 61 μg L^{-1}), 46% from supplied tap water (median As = 53 μg L^{-1}), and the rest from private tube wells. Comparing the As content of this block to the Chadka block of Nadia reveals that three times the amount of As exists in the Bhawangola-I block of the Murshidabad district (Mondal et al., 2010). More quantitatively, 61.2% of samples collected by SOES exceeded 10 μg L^{-1} (WHO standard) and 30.6% exceeded 50 μg L^{-1} (Indian standard) (SOES, 2006).

Murshidabad also experiences distinct seasonal changes in As concentrations. Farooq at el. (2011) found that As content decreased as the seasons changed from premonsoon (mean As = 63.2 μg L^{-1}) to monsoon (mean As = 59.2 μg L^{-1}) to postmonsoon (54.9 μg L^{-1}). This same trend persisted for almost all other elements, and is further discussed in the "Seasonal/Temporal Variations" section.

It became evident in Murshidabad that perennial ponds are not the main contributing source of water or dissolved organic matter responsible for high As. Stable isotope values from values for δ^2H and $\delta^{18}O$ indicate that groundwater is directly recharged by local precipitation without significant evaporation and therefore not mixed or recharged by pond waters. The travel time for pond dissolved organic matter surpasses that of groundwater by thousands of year, as seen in reactive transport modeling (Datta et al., 2011).

Sankar et al. (2014) investigated the extent and release mechanisms of As and manganese (Mn) contaminations in Murshidabad. Alarming levels of As, in relative coherence with Mn, were found in distinct zones: High As, low Mn; High Mn, low As. High Mn, low As zones were explained by the idea that subsurface redox conditions were reducing enough to promote Mn(III) reduction and therefore releases Mn(II), however not quite reducing enough to permit the reduction of Fe(III) oxyhydroxides and subsequent release of As. The high As, low Mn zones exhibited redox conditions conducive to both Mn(III) and Fe(III) reduction, however due to a slightly more alkaline nature, it is suggested that $MnCO_3$ was in fact supersaturated and therefore separating out of the aqueous phase in the form of rhodocrosite precipitation.

3.1.3 *Malda*

Malda is bordered by Bangladesh to the east, Murshidabad to the south and Bihar to the North, covering an area of 3733 km^2. 3,290,468 people live in 1642 villages distributed in 15 blocks (SOES, 2006). The area of Malda is generally flat and represents a part of the vast alluvial plain with elevation ranging from 22.4 to 25.3 m (Sikdar and Chakraborty, 2008). A progression of terraces makes up the area and they are generally 1 m high from each other. The geology of the region consists of sand, silt, and clay cycles with carbonaceous matters and carbonate and ferruginous concretions present in some areas (Purkait et al., 2008). The Ganga, Mahananda, and Kalindri rivers drain the area. 4449 tube wells were surveyed and it was determined that 52.2% > 10 μg L^{-1}, 34% > 50 μg L^{-1}, and 6.3 > 300 μg L^{-1}. The maximum As concentration was 1904 μg L^{-1} (SOES, 2006).

For the majority of the Malda district, the subsurface aquifer is the main source of drinking water. Groundwater in the Malda district is plentiful and generally occurs in a thick zone of saturation within the alluvial sediments. Madhavan and Subramanian (2006) took a cross-section of an aquifer between the Ganga and Mahananda Rivers. The general flow of the groundwater is south to southwest, which is consistent with the slope of the area. As concentration was highest in the south and southwest (Ray and Purkait, 2010). Madhavan and Subramanian (2006) found the amount of Holocene clay over Holocene sand is proportional to redox potential of groundwater. Overall, it was observed that groundwater in the majority of Malda was found to have less-oxidized redox potential. The redox potential is less oxidized when Holocene clay over Holocene sand increases (15–18 m). When the clay over sand decreases (9–2 m), groundwater proves to be more oxidized (Madhavan and Subramanian, 2006).

As concentrations of four blocks in the Malda district were extensively studied by Sikdar and Chakraborty (2008): Kaliachak-I (mean As = 225.3 μg L^{-1}); Kaliachak-II (mean As = 192.3 μg L^{-1}); Kaliachak-III (mean As = 175.5 μg L^{-1}); and English Bazar (mean As = 132.4 μg L^{-1}). The steady decrease in concentrations of these blocks is consistent with the geological conditions described in the previous paragraph. The dissolved cation content in the groundwater of the Malda district was found to be a result of 79% mineral dissolution and 21% anthropogenic activity (Sikdar and Chakraborty, 2008).

3.1.4 Burdwan

Burdwan (also called Bardhaman) is located south of Murshidabad, west of Nadia, and North of Hooghly, covering an area of 7024 km^2. The district of Burdwan is a dominant agricultural producer and is known as the granary of West Bengal. 6,919,698 people live in 2488 villages encompassing 32 blocks. 2,634 tube wells were analyzed and the following data were collected: 17.6% > 10 μg L^{-1}, 8.3% > 50 μg L^{-1}, and 0.7% > 300 μg L^{-1}. The maximum concentration observed was 2230 μg L^{-1} (SOES, 2006).

In the Purbasthali block, Biswas (2010) examined 40 villages for As content. The results showed that 100% > 10 μg L^{-1}, 70% > 50 μg L^{-1}, and 20% > 90 μg L^{-1}. One village called Mandra had values exceeding 300 μg L^{-1}, and has displayed the worst cases of arsenicosis. Purbasthali is located in central Burdwan and is the most contaminated block in the district. Biswas (2010) also found that As concentration positively correlated with surface water coverage, indicating that geomorphic features play a vital

role in As cycling. Well depth did not seem to have significant impact on As, however. Previously, Nag et al. (1996) performed a similar study, noting high concentrations of Fe (300–1007 μg L^{-1}) and moderate Sb (0.03–0.9 μg L^{-1}).

The geology of the majority of Burdwan district is characterized by younger and older Tertiary to Quaternary age alluvium, with lower Gondwana sedimentary rocks classifying the western part. The western part suffers from water shortage, whereas the east has considerable groundwater supplies. The majority of groundwater in the area is alkaline in nature. Gupta et al. (2008) found most of the Burdwan district to have medium salinity–low sodium adsorption ratio, with some areas having high salinity and high sodium adsorption ratio (Gupta et al., 2008).

3.1.5 Hooghly

Hooghly is located to the south of Burdwan, west of Nadia, and 24 Parganas North and north of 24 Parganas South. It covers an area of 3149 km^2 and contains 18 blocks. 5,041,976 people live in 1886 villages in the district. 2212 tube wells were surveyed and 17.9% > 10 μg L^{-1}, 6.6% > 50 μg L^{-1} and only three tube wells exceeded 300 μg L^{-1} with no samples recording over 1000 μg L^{-1} (SOES, 2006). To date, there are very few publications pertaining to As contamination in Hooghly district. The extent to which As pollutes this region will likely be further examined in the future.

3.1.6 Howrah

Howrah lies to the south of Hooghly and to the west of 24 Parganas South, across the Ganga from Kolkata. It covers an area of 1467 km^2 and is divided into 14 blocks. 727 villages house the 4,273,090 residents of the district. 1471 tube wells were surveyed, which revealed that 24.2% > 10 μg L^{-1}, 11.1% > 50 μg L^{-1} with only 14 tube wells exceeding 300 μg L^{-1}. The maximum As concentration was 1333 μg L^{-1} (SOES, 2006). Similarly to Hooghly, Howrah is one of the least studied districts of West Bengal. The distribution of As in Howrah will need to be studied in more detail in order to fully quantify and address the globally known issue that is As contamination.

3.1.7 24 Parganas North

This district is located in the southern portion of the Bengal Basin: east of the Ganga River, south of Nadia district, and west of Bangladesh. It comprises

4094 km^2 and is divided into 22 blocks, with 8,934,286 people living in 1582 villages. 54,368 tube wells were surveyed and it was determined that 53.4% > 10 μg L^{-1}, 29.5% > 50 μg L^{-1}, and 3.4% > 300 μg L^{-1}. 49 tube wells had As concentrations above 1000 μg L^{-1} and the maximum concentration was 2830 μg L^{-1} (SOES, 2006).

According to Roychowdhury (2010), p. 20 of the 22 blocks in 24 Parganas North are affected by As contamination (mean As > 50 μg L^{-1}). Arsenic-related skin lesions have been detected in 16 blocks. 3,061 hand tube wells were sampled in various gram panchayats in Gaighata block, and 40.8% < 10 μg L^{-1}, 18.9% between 10 and 49 μg L^{-1}, 15.6% between 50 and 100 μg L^{-1}, 21.2% between 100 and 300 μg L^{-1}, 2.71% between 300 and 500 μg L^{-1}, 0.58% between 500 and 700 μg L^{-1}, and 0.19% between 700 and 1000 μg L^{-1}. The worst of these is Gutri mouza of Ischhapur II (94.7% > 50 μg L^{-1}). In all of Gaighata block, approximately 106,560 people (41%) drink As contaminated water >50 μg L^{-1} (Roychowdhury, 2010). A number of biological samples were also taken, and 93% maintained As levels above average. 68% of urine samples exceeded 200 μg L^{-1}, whereas the normal range lies between 3.33 and 26.7 μg L^{-1}. 174 of 930 people screened for arsenical dermatosis were deemed As patients. 64% of surveyed areas found >80% of sampled hair to have As content above normal; 82% found >80% of sampled nails to have As content above normal.

Roychowdury (2010) also found a correlation between Fe and As contamination. Roughly 98% of the sampled water displayed Fe content (>300 μg L^{-1}) deeming a taste "unacceptable" by the World Health Organization. It was noted that both As and Fe tend to gradually decrease with depth.

3.1.8 24 Parganas South

24 Parganas South is the southernmost district of West Bengal, lying just east of the Ganga River, and is bordered to the south by the Bay of Bengal. The River Malta drains the southern parts of 24 Parganas South. It covers 9960 km^2 and is divided into 29 blocks. 6,906,689 people live in 2087 villages in the district. 8334 tube wells have been surveyed and it has been determined that 42% > 10 μg L^{-1}, 28.3% > 50 μg L^{-1}, and 6.6% > 300 μg L^{-1}. 30 tube wells contained >1000 μg L^{-1} As with the maximum concentration detected at 3700 μg L^{-1} (SOES, 2006).

A seasonal As variation study was conducted by Savarimuthu et al. (2006). Four police stations (Sonarpur, Baruipur, Bhangor, and Mograhat) were examined, covering 5% of the total 24 Parganas South land area.

Tube well samples revealed a noticeable increase during the Monsoon season of 2003 in comparison to the Summer and Winter seasons of that same year. Similar data for Murshidabad was also noted (Farooq et al., 2011). Savarimuthu et al. also found a significant increase in As concentration (+335 μg L^{-1}) between Winter 2002 and Winter 2003. The only speculation for this behavior was the possible influence of the sharp decline in rainfall between the two seasons (−537 mm), although no specific mechanism has been able to fully explain this change.

3.2 Uttar Pradesh

Uttar Pradesh is located in the upper and middle Gangetic Plain in northeast India, and is the most heavily populated area of this region (Kumar et al., 2010a). It is bordered by Nepal to the north, the Indian state of Bihar to the east, and Madhya Pradesh to the south. Figure 3 displays the various

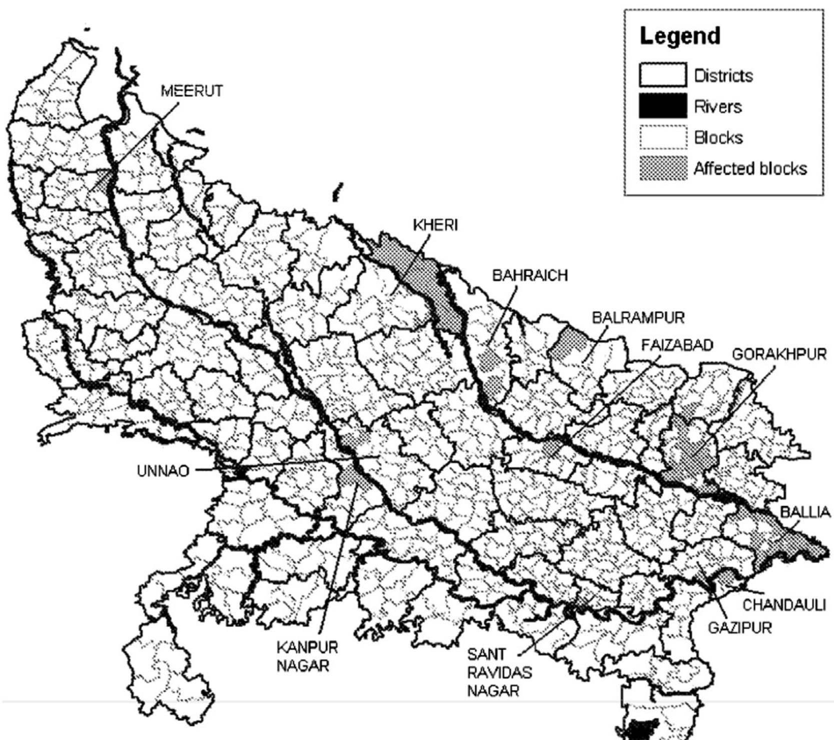

Figure 3 As affected blocks in Uttar Pradesh. *As adapted from Nickson et al. (2007). Reprinted by permission of the publisher Taylor & Francis Ltd, http://www.tandfonline.com.*

As affected blocks within Uttar Pradesh (Nickson et al., 2007). The district has varied topography due to many streams and is a peneplain. The area comprises of Quaternary age alluvium with successions of clay, clayey silt, and sand (Raju, 2012). 166 million people live in 70 districts that divide the 238,000 km^2 state. As was first detected in groundwater in Uttar Pradesh in 1976 (Ahamed et al., 2006). Since then, As has been detected in tube wells from the eastern part of the state. 4780 tube wells have been surveyed for As contamination in three eastern districts (Ballia, Gazipur, and Varanasi) by Ahamed et al. (2006). It was determined that 45.5% > 10 μg L^{-1}, 26.5% > 50 μg L^{-1}, and 10% > 300 μg L^{-1} 11 villages were screened for As skin lesions, and 154 of 989 people were found to be affected, and 32 of 74 people examined were diagnosed with arsenical neuropathy (Ahamed et al., 2006).

Ghazipur district in east Uttar Pradesh was studied extensively by Kumar et al. (2010a). The geology consists of primarily Gangetic-Karamnasa alluvial sediments and makes up the eastern portion of the peneplain. 84% of the area is used for agricultural purposes, with only surface water and tube wells as sources of irrigation. 30 samples were collected in premonsoon and postmonsoon season of 2007. The pH remained relatively constant, averaging 7.8 (pre) and 7.7 (post). Premonsoon samples displayed an overall negative charge imbalance with ions mostly in Ca-Cl, Ca-SO_4, and Mg-SO_4-Cl facies. Postmonsoon samples exhibited an overall positive charge imbalance and predominantly Ca-Mg-HCO_3 facies. Possible causes of charge imbalance include failing to analyze organic matter, although no direct cause was identified. As concentrations were once again lower in postmonsoon (mean As = 38.3 μg L^{-1}) compared to premonsoon (mean As = 48.4 μg L^{-1}), and generally higher in As(III) than As(V). Notable villages were Devbaranpur (259.5 μg L^{-1}), Ghazipur City (222.8 μg L^{-1}), and Rajapur (173.7 μg L^{-1}), all of which lay along the Ganga floodplains (Kumar et al., 2010a). The hydrogeochemical details of this investigation are further discussed in the "Seasonal/Temporal Variations" section of this review.

The Ganga plain is known to contain more sand and less clay from the Quaternary deposits, which occupy the middle of the plain. This indicates less retention of As and thus the extent of As contamination in the middle of the Ganga plain is less than the Bengal Delta (Raju, 2012). Raju (2012) studied the middle Ganga plain (about 100 km^2) and found overall lower As due to its position in Pleistocene older alluvium upland surfaces. Lower values of dissolved iron were observed in the middle of the Ganga plain

(<0.7 mg L^{-1}) compared to West Bengal (up to 36 mg L^{-1}). Additionally, low concentration of HCO_3^- was observed between 10 and 20 m depth (Raju, 2012).

A similar study was performed by Shah (2010a), and As content was found to be generally highest in entrenched channels and floodplains of Holocene Newer Alluvium sediments. These sediments are gray to black in color, organic-rich, and argillaceous. The highest As concentration of this study was found in Singhaour (mean As = 180 μg L^{-1}), a small village that lies along Holocene Newer Alluvium sediments just west of the Ganga River. Pleistocene Older alluvium sediments are essentially free of As contamination, and are characterized by yellowish-brown sediments with ferruginous and calcareous concretions (Shah, 2010a,b). Shah (2010a) also examined Fe concentrations, and discovered that 83% of tube well samples exceeded the permissible limit of 1 mg L^{-1}. Fe and As were determined to be positively correlated.

Kanpur-Unnao region, within Uttar Pradesh, is one of the largest industrial areas in the Ganga Plain. Unnao-Kanpur situated between the Ganga River and its tributary, the Padu River. Chauhan et al. (2011) studied the groundwater of Shuklaganj area of Kanpur-Unnao where they found most samples contained both As(III) and As(V). Overall, they found the concentration of As(III) to be higher than As(V). The dominant ions in the groundwater were HCO_3^- and Na^+ and the pH of the groundwater was found to be slightly alkaline. They observed a positive correlation of As observed with NH_3, PO_4^{3-}, and Fe, whereas a negative correlation with nitrate and sulfate (Chauhan et al., 2011).

Nepal borders Uttar Pradesh just to the north and elevated levels of As have been found in the drinking water as well. Results indicate that As levels are not as high, although about 13% exceed WHO drinking water standards. Concentrations of As were $<10-1200$ μg L^{-1} in groundwater from tube wells at 7–55 m depth. One outlier was found in Rupandehi district of Nepal with a concentration of 2620 μg L^{-1}. Elevated levels of As in Nepal is similar to that of the Bengal Delta region in that it is mainly due to the reduction of Fe-oxyhydroxides in the presence of organic matter (Tandukar et al., 2005).

3.3 Bihar

Bihar is located in the middle Gangetic Plain and covers an area of 94,163 km^2 with a population of 8.2 million. Groundwater chemistry in the Gangetic plain area reflects a greater variety of differences in the

geomorphological and geological setting of the aquifers (Mukherjee et al., 2012). The Ganga River flows through the state from west to east. Bihar has been receiving attention due to the fact that 12 districts and 32 of its blocks on either side of the Ganga River have been found to be affected by As pollution (Chaturvedi et al., 2010). Figure 4 depicts the blocks affected by As contamination (Nickson et al., 2007). The Holocene aggradations are more pronounced in the Ganga plains of Bihar compared to Uttar Pradesh, which is largely due to rising base level and climate-driven sediment supply (Kumar et al., 2010a). As was surveyed in 19,961 tube well samples and 32.7% $> 10\ \mu g\ L^{-1}$, 17.75% $> 50\ \mu g\ L^{-1}$, 4.5% $> 300\ \mu g\ L^{-1}$ and the maximum concentration was 2100 (SOES, 2006). As was surveyed in Semria Ojha Patta village in the Bhojpur district located in western Bihar. Semria. Ojha Patta is an agrarian village with no industry nearby. 200 tube wells were sampled and it was found that 24.7% $> 10\ \mu g\ L^{-1}$, 56.8% $> 50\ \mu g\ L^{-1}$ and 19.9% $> 300\ \mu g\ L^{-1}$ (Chakraborti et al., 2003). In Rampur Diara (mean As $= 52\ \mu g\ L^{-1}$) and Haldichapra (mean As $= 231\ \mu g\ L^{-1}$), panchayats of Maner block in Patna district, 100% of water samples exceeded 50 $\mu g\ L^{-1}$ (Singh and Ghosh, 2012). Cancer risk in children was found to be 19 per 1000 (Rampur Diara) and 87 per 1000 (Haldichapra), and calculated daily consumption of As was

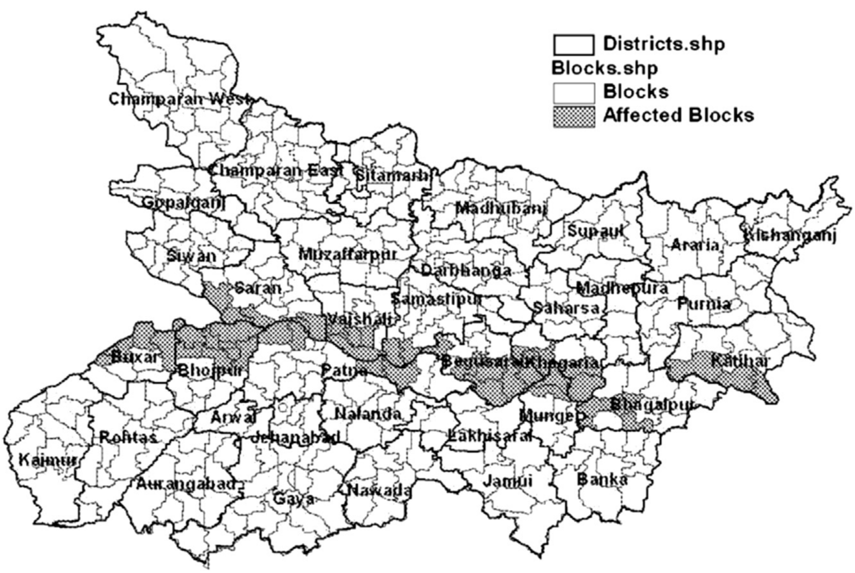

Figure 4 As affected blocks in Bihar, India. *Reproduced from Nickson et al. (2007). Reprinted by permission of the publisher Taylor & Francis Ltd, http://www.tandfonline.com.*

120 μg day^{-1} (Rampur Diara, 5–10 years old), 320 μg day^{-1} (Rampur Diara, >41 years old), 580 μg day^{-1} (Haldichapra, 5–10 years old), and 1470 μg/day (Haldichapra, >41 years old) (Singh and Ghosh, 2012). All of the contaminated tube wells lie in the Present Day and Older Flood Plain surfaces ranging from (>50 to <750 ppb) (Chaturvedi et al., 2010).

In this flood-prone area, organic matter is carried by rainfall infiltration from recently accumulated biomass. This process plays an important role in releasing As and iron into the sediments in the shallow aquifers (Saha, 2009). Aquifers containing As contaminated groundwater occur within the narrow oscillation zone of the Ganga River. The maximum thickness of the Holocene sediments deposited by the Ganga River within oscillation zone is 70–80 m. Below 70–80 m is the Pleistocene sediments where groundwater is free from As (Chaturvedi et al., 2010). Older alluvium, (Pliestocene deposits) have lower HCO_3 levels, more alkaline levels and less As than newer alluvium (Holocene). The iron concentration of newer alluvium is five times greater than older alluvium (Saha, 2009). Chaturvedi et al. (2010) sampled 20 surface water samples from streams and found all to be free from As.

The groundwater beneath Bihar is characterized by an abundance of Ca^{2+}, Na^{+}, K^{+}, and NH_4^+ cations, in order of decreasing abundance. Prevalent anions include Cl^{-}, SO_4^{2-}, NO_3^-, and PO_4^{3-}. As was found to correlate strongly with Fe, PO_4^{3-}, and NH_4^+ (Kumar et al., 2010b). The majority of As contamination occurs near the Ganges River, implying that fluvial geomorphology plays a key role in As distribution. A distinct layer of fine-grained clay between 4.5 and 7.5 m deep aids in the vertical distribution of As. 66% of total As was attributed to the trivalent species (Kumar et al., 2010b). Saha et al. (2011) used isotopic signatures to further characterize groundwater behavior of Bihar region, and this study is extensively discussed in the section of this paper titled, "Hydrology."

3.4 Jharkhand

Jharkhard, part of the Gangetic basin, encompases 15,499 km^2 and has a population of approximately one million people (SOES, 2006). Compared to the terrains of the Bengal Basin downstream, the districts of Bihar and Jharkhand have more distinctive geologic and geomorphic terrains. This is due to the meandering and avulsing rivers. The terrains are also stacked over each other (Mukherjee et al., 2009). Lower amounts of groundwater abstraction occur in this area compared to the Bengal Basin. In Jharkhand, the Sahibgunj district which borders the district of Malda in West Bengal

has been surveyed as of 2007 (SOES, 2006; Nickson et al., 2007). 32.8% of wells tested has As concentrations >10 μg L^{-1}, 13.44% > 50 μg L^{-1} and 2.61 > 300 μg L^{-1} (SOES, 2006). These data do not represent the entire state, but only the district of Sahibgunj and it is possible that the contamination could be more widespread (Nickson et al., 2007). In Majhiaon block of Garwa district, fluoride also poses a serious contamination issue, as 402 of 840 samples taken exceeded the permissible limit (Avishek et al., 2010). Figure 5 provides a map depicted the various As affected blocks within Jharkhand (Nickson et al., 2007).

Bhattacharjee et al. (2005) studied the area of Sahibgunj, which is located on the western bank of the Ganges River. They found 66–90% of the tube wells surveyed to contain toxic amounts of iron in the water. High manganese was also found for the Sahibgunj area, indicating reductive dissolution of oxyhydroxides of manganese and iron as a factor of As mobilization (Bhattacharjee et al., 2005). Mukherjee et al. (2012) found that redox-sensitive parameters indicate reducing conditions with no ordered depth variation from the sampled aquifers. Groundwater was of Ca-HCO_3^- composition.

The Gangetic basin is important to compare to the Bengal Basin because it hosts some of the most bountiful aquifers. In this area, there is less water

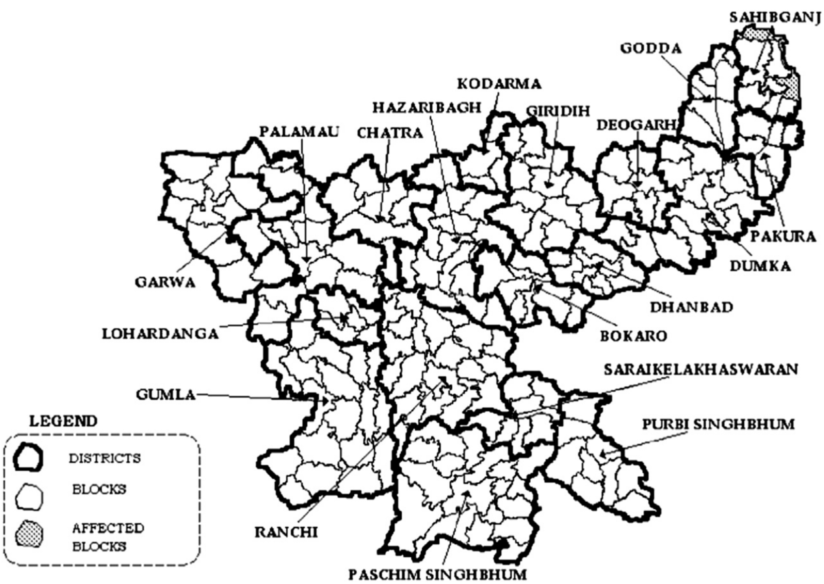

Figure 5 As affected blocks of Jharkhand. *Reproduced from Nickson et al. (2007). Reprinted by permission of the publisher Taylor & Francis Ltd, http://www.tandfonline.com.*

extraction and more distinctive geologic and geomorphic terrains. The terrain of Jharkhard includes pre-Cenozoic-aged metamorphics and volcanics, older alluvial deposits from the Ganges River and its tributaries, and younger alluvial deposits from the Himalayas. Groundwater from the alluvial sediments of the tributaries of the Ganges River indicates a relationship between As and competitive anions, which is proposed to be a result of agriculture or water–rock interaction (Mukherjee et al., 2012).

A number of health problems have arisen in the Sahibgunj district of Jharkhand (42% tube wells >300 μg L^{-1}). Nayak et al. (2008) obtained 367 biological samples (hair, nail, urine) and found that 88% exceeded the normal level of As. As a result, As skin lesions were common, with 71 of 522 people screened exhibiting some form of As-related skin lesion. It should be noted that the ratio in just children was higher: 9 out of 40 (Nayak et al., 2008).

3.5 Assam

After As was first detected in Assam in 2004, the SOES analyzed 241 tube well samples for As contamination (SOES, 2006). Analyses revealed that 42.3% of tube wells had As >10 μg L^{-1} and 19.1% > 50 μg L^{-1} (SOES, 2006). These samples were taken from the Dhemaji and Karimganj districts. Most of the As-affected villages are located close to abandoned or present meander channels of Barak, Surma, and Langai Rivers (Shah, 2012). Further sampling in 22 districts supported by the UNICEF "rapid assessment" program found that 18 districts are affected by As contamination with 6.3% > 50 μg L^{-1} As (Nickson et al., 2007). In 2009, Mahanta et al. conducted a similar survey, however it was found that 72 out of 192 blocks contained As levels >50 μg L^{-1}. Golaghat district of Assam was specifically studied by Chetia et al. (2009). The district is located in central Assam and is bounded by Karbi-Anglong district to the west and Jorhat and Nagaland districts to the east. River Kakodonga forms the eastern border connecting to Jorhat, and flows south to north. The Dhansiri River flows from southeast Golaghat north until it meets River Brahmaputra, which continues throughout the northern portion of the district. Golaghat is thought to contain between three and nine aquifer systems, with average water level depths between 2 and 7 m. The aquifers in the northern region are unconfined while progressing south reveals confinement. 222 water samples were taken in spring of 2008, and As, Fe, and Mn were all found to be contaminants (Chetia et al., 2009). As data showed that three blocks were of particular threat: Gamariguri (100% As > 10 μg L^{-1}), Podumoni

(87.5% As > 10 μg L^{-1}), and Kathalguri (71.9% As > 10 μg L^{-1}). Fe analysis displayed that 76.4% of samples taken exceeded 1000 μg L^{-1} (WHO limit), and Mn analysis showed that 28.5% of samples contained >300 μg L^{-1} (WHO limit). Gamariguri block was most severely affected by all three elements. Throughout the district, As contamination is most prevalent in younger alluvium sediments, and 78% of As > 10 μg L^{-1} was found at 180 ± 10 ft depth (Chetia et al., 2009). These surveys of Assam do not cover all blocks throughout the districts, however, and thereby provide an unclear picture of the extent of As contamination in Assam (Nickson et al., 2007).

Assam is located in a hilly region of northeast India. The southern part of Assam is made up of the Barak Valley. In the Barak Valley, tube wells located in the Plio-Pleistocene aquifers contain less levels of As compared to the Holocene Newer Alluvium. Tube wells polluted with As are located in the lowland valley areas with cover of Holocene Newer Alluvium sediments on top (Shah, 2012). Figure 6 displays the various As affected blocks within Assam (Nickson et al., 2007).

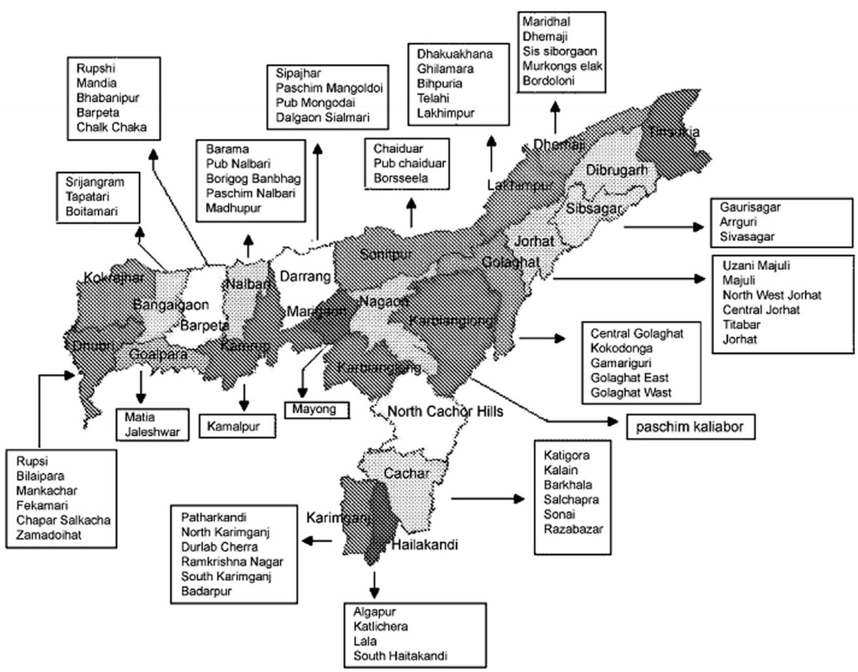

Figure 6 As affected blocks in the Indian state of Assam. *Reproduced from Nickson et al. (2007). Reprinted by permission of the publisher Taylor & Francis Ltd, http://www.tandfonline.com.*

3.6 Chhattisgarh

Chhattisgarh is located in central India and is not situated in the Bengal Basin. For this reason, its geological, hydrological, and geochemical mechanisms for As release and contamination are different from contaminated sites within the basin. This area has shallow, thin alluvium and soil cover, and groundwater wells tap fractured bedrock aquifer (Acharyya et al., 2005). There are four villages that contain As levels above 50 μg/l and frequently contain over 100 μg L^{-1}. These villages are Kaurikasa Sonsayatola, Joratarai, and Jadutola. Mine dumps have been reported near the severely affected village of Kaurikasa, which could be contributing to the As contamination (Acharyya et al., 2005). The geology and As mobilization mechanisms in this Indian state will be discussed below.

Kaurikasa, Sonsayatola, Joratarai, and Jadutola all exist in the Ambagarh-Chowki block of Chhattisgarh. As contamination in this region is restricted to the Dongargarh rift zone, which consists of acid and basic metavolcanics and comagmatic and contemporaneous Dongargarh Granite, and geographically points in the N–S direction (Acharyya et al., 2005). Majority of As polluted areas tend to exist on acid volcanics near shear zones rather than granite, with the exception of Sonsayatola. The other three prominent villages all exist on acid volcanic bedrock, prominently rhyolite. Although pyrite is a common accessory mineral in shear zones, its oxidation is not a common source of As in this region. Most As actually occurs as adsorbed particulate matter or coatings on clay and silicate minerals in HFO (hydrated Fe oxide) phases. Fe-reducing microbes often reduce these phases, releasing Fe and sorbed As to solution and causing increased levels of these elements in groundwater. The primary source of As, however, is likely the weathering and erosion of clay and silicate minerals (Acharyya et al., 2005).

4. ARSENIC GEOCHEMISTRY

Arsenic is the 20th most abundant trace metal on earth and occurs in a variety of environments (Duker et al., 2005). It is a toxic element that can have detrimental health effects, if ingested (Chakraborti et al., 2003). It can be associated with minerals susceptible to weathering, such as sulfides, which allows it to enter the environment where it has caused substantial health problems in 35 countries throughout the world (Duker et al., 2005; Das et al., 2012). As concentrations are highest in groundwater where the effect of water–rock interactions release As into solution and

geochemical conditions allowing As mobilization are present (Smedley and Kinniburgh, 2002). A complete review of the geochemistry of As is beyond the scope of this paper, but a general overview will be given to help readers understand the key issues related to As contamination in India and Bangladesh. For further details refer to Smedley and Kinniburgh (2002), Cullen and Reimer (1989) and Onishi and Sandell (1955).

Arsenic can occur in several valence states in the natural environment: −3, 0, +3, and +5 (Smedley and Kinniburgh, 2002). It commonly occurs as As^{3+} (arsenite) and As^{5+} (arsenate) as inorganic oxyanions, with arsenite (As^{3+}) as the dominant species in reducing environment, while arsenate (As^{5+}) as dominant species at oxidizing conditions (Smedley and Kinniburgh, 2002) (Figure 7). It can also occur in a range of organic forms due to microbial activity, but their relative abundance is low (Smedley and Kinniburgh, 2002; Bhattacharya et al., 2002a). Arsenic can be mobilized at circumneutral pH values (6.5−8.5) typical of fresh groundwater (Smedley and Kinniburgh, 2002). Oxyanions, such as arsenate, are less able to be sorbed at higher pH in

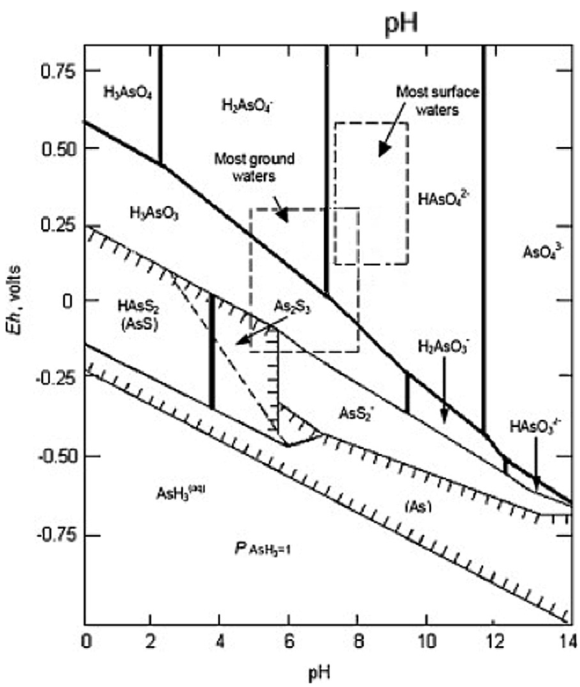

Figure 7 Eh-pH diagram for arsenic species in water (Schnoor, 1996). *Jerald L. Schnoor, Robert C. Ahlert, Environmental Modeling: Fate and Transport of Pollutants in Water, Air, and Soil. John A. Wiley & Sons, Inc.*

contrast to other heavy metals, which tend to precipitate out of solution at high pH values (Smedley and Kinniburgh, 2002). As is also sensitive to changes in redox conditions in groundwater. Unlike most oxyanions, As is most mobile under reducing conditions (Smedley and Kinniburgh, 2002).

Arsenic can occur in over 200 minerals (sulfides, oxides, arsenides, arsenites, and arsenates) but is most commonly found in arsenoprite, FeAsS (Smedley and Kinniburgh, 2002). Table 2 shows common As containing

Table 2 Major minerals containing arsenic (Smedley and Kinniburgh, 2002)
Major As minerals occurring in nature

Mineral	Composition	Occurrence
Native arsenic	As	Hydrothermal veins
Niccolite	NiAs	Vein deposits and norites
Realgar	AsS	Vein deposits, often associated with orpiment, clays and limestones, also deposits from hot springs
Orpiment	As_2S_3	Hydrothermal veins, hot springs, volcanic sublimation products
Cobaltite	CoAsS	High-temperature deposits, metamorphic rocks
Arsenopyrite	FeAsS	The most abundant As mineral, dominantly in mineral veins
Tennantite	$(Cu, Fe)_{12}As_4S_{13}$	Hydrothermal veins
Enargite	Cu_3AsS_4	Hydrothermal veins
Arsenolite	As_2O_3	Secondary mineral formed by oxidation of arsenopyrite, native arsenic and other As minerals
Claudetite	As_2O_3	Secondary mineral formed by oxidation of realgar, arsenopyrite and other As minerals
Scorodite	$FeAsO_4 \cdot 2H_2O$	Secondary mineral
Annabergite	$(Ni, Co)_3(AsO4)_2 \cdot 8H_2O$	Secondary mineral
Hoernesite	$Mg_3(AsO_4)_2 \cdot 8H_2O$	Secondary mineral, smelter wastes
Haematolite	$(Mn, Mg)_4Al(AsO_4)(OH)_8$	
Conichalcite	$CaCu(AsO_4)(OH)$	Secondary mineral
Pharmacosiderite	$Fe_3(AsO_4)_2(OH)_3 \cdot 5H_2O$	Oxidation product of arsenopyrite and other As minerals

minerals and their occurrence. As can also be present as a trace element in rock-forming minerals such as pyrite, marcasite, chalcopyrite, and galena (Smedley and Kinniburgh, 2002). It commonly substitutes for S in sulfide minerals and pyrite is an important mineral that influences As occurrences in groundwater (Smedley and Kinniburgh, 2002). As is present in igneous and metamorphic rocks, but is generally at higher concentrations in sedimentary rocks (Smedley and Kinniburgh, 2002). Recent digestion studies of mica minerals from the Bengal Basin suggest that they are an important source of As in the Bengal Basin aquifers (Dowling et al., 2002). Abundant mica has been observed in sandy sediments from West Bengal, India (Rowland et al., 2006). Holocene sediments deposited in the Bengal Basin and Ganges plain are derived from igneous and metamorphic rocks weathered in the Himalayan Mountains (Mukherjee et al., 2009). These sediments contained oxide minerals with adsorbed As and are thought to be the source of As that has contaminated this area (McArthur et al., 2001; Saunders et al., 2008).

In sediments, As is associated with Al, Fe, and Mn oxide minerals and is commonly found bounded to their surfaces (Smedley and Kinniburgh, 2002). In freshwater aquifers Fe oxides act as an important As adsorber because of their high abundance (Smedley and Kinniburgh, 2002). Fe oxides are common in the aquifers of the Bengal Basin and Ganges Plain and have been associated with As (Nickson et al., 2000). As has a higher affinity for Fe oxides over Mn oxides, which has been shown experimentally (Ying et al., 2012). As adsorbed onto the oxide surfaces can be mobilized into the environment if the correct geochemical conditions are present.

Three mechanisms have been proposed to account for the mobilization and accumulation of As in groundwater in the Bengal Basin. First, the oxidation of As-bearing pyrite (Mandal et al., 1996; Mallick and Rajagopal, 1996) has been largely dismissed as the main mechanism of release due to the lack of substantial pyrite in the sediments (Umitsu, 1993), strongly reducing conditions prevalent in the contaminated aquifers (von Bromssen et al., 2007), and lack of dissolved sulfate present in groundwater (Acharyya et al., 1999). The second mechanism involves the reductive dissolution of Fe-oxyhydroxide minerals with sorbed As (McArthur et al., 2001; Bhattacharya et al., 1997; Nickson et al., 2000). This is the prevailing theory to explain the high As concentrations in Bengal Basin aquifers and will be discussed in detail. The third mechanism is the introduction of P from fertilizer and organic matter decomposition and competitive exchange of As for P on Fe-oxyhydroxide surfaces (Acharyya et al., 1999). The quantities of P

required to mobilize the amount of As observed is unrealistic, given the depth of aquifers and the relative nonmobility of P through sediments (Bose and Sharma, 2002). It is probable that several mechanisms are responsible for As mobilization and release into Bengal Basin groundwater.

Arsenic is mobilized into aqueous solution in geochemically reducing conditions (Cullen and Reimer, 1989). When Fe-oxyhydroxides are reduced, any As bound to the surface is released into the water (Smedley and Kinniburgh, 2002). The reduction of Fe-oxyhydroxides and sulfates can be controlled by microbial activity. Experimental studies have found that microbes play an important role in speeding up the reductive dissolution of Fe-oxyhydroxides and the subsequent release of As (van Geen et al., 2004). Sediments from Bangladesh were incubated with an antibiotic and minimal reductive dissolution and release of As was recorded (van Geen et al., 2004.) Another experiment examined the role of microbes in sediment from West Bengal, India and found that the microbes facilitated the reduction of Fe and released As (III) into solution (Islam et al., 2004). Correlations between As concentrations with iron, methane, and ammonia in aquifers in Bangladesh are further evidence of microbial reduction of Fe oxides (Dowling et al., 2002). Models have suggested that microbial mediated reduction of Fe-oxyhydroxides can introduce As at a rate of 1.8 uM year^{-1} (Dowling et al., 2002).

Fe-oxyhydroxides are favorable over sulfate as a terminal electron acceptor (Saunders et al., 2008). Sulfate reduction takes over after sufficient ferric species have become depleted and conditions become more reducing (Saunders et al., 2005; Lee and Saunders, 2003). It has been proposed that when sulfate reduction overtakes Fe reduction, sulfide species will form and trap As in the crystal structure (Saunders et al., 2008). If sufficient Fe and sulfides are present, authigenic pyrite will form from amorphous Fe-S compounds (Morse and Cornwell, 1987). As can be captured by these phases and incorporated into the crystal structure of pyrite (Saunders et al., 2008). This development of redox drivers could serve to trap As over time and when ferric Fe species are depleted. As up to 1 wt% has been found in authigenic pyrite, suggesting this trapping mechanism is viable (Saunders et al., 2008).

Experimental work in As-rich aquifers in the United States has found that while sulfate-reducing bacteria are active, As is removed from solution and stays trapped after bacterial sulfate reduction has ceased (Saunders et al., 2008). The same experiment conducted in Bangladesh had similar results, except As concentrations increased after sulfate reduction cease

(Saunders et al., 2008). This suggests that the ability of the Bengal Basin aquifers to trap As during sulfate reduction is limited. This is supported by studies of As mobilization in Bangladesh where sulfate reduction was not trapping As in authigenic sulfides (Zheng et al., 2004). Low sulfate concentrations in the groundwater did not allow for the formation of enough sulfide minerals to trap significant quantities of As (Zheng et al., 2004).

The mobilization of As by the reductive dissolution of Fe-oxyhydroxides has been challenged by some who argue the As is mobilized up gradient and is transported through the aquifer system (Polizzotto et al., 2005). As concentrations peak around 30 m depth in gray, reduced sediments that do not contain significant sources of Fe-oxyhydroxide minerals to sustain high concentrations of As (Polizzotto et al., 2005). The presence of methane suggests the process of Fe reduction has ceased and flushing of the aquifer would be expected to reduce the concentration of As at this depth. Polizzotto et al. (2005) argue that the bell-shaped distribution of As and associated species are typical of a contaminant plume migrating through an aquifer. Organic carbon from the surface could migrate into the shallow aquifer where changing redox conditions allow for the release of As from sediments (Polizzotto et al., 2005).

Sources of organic carbon from the surface are perennial ponds, pit latrines, septic systems, and other sanitation methods (McArthur et al., 2012; Datta et al., 2011). Stable isotopic investigations of pond water indicate they are recharged during the monsoon season and undergo extensive evaporation in the dry seasons (Datta et al., 2011). These signatures are not found in aquifer groundwater, indicating they are directly recharged by precipitation and not surface infiltration from ponds (Datta et al., 2011). In addition, reactive transport models of organic carbon from ponds indicate transport times significantly longer than groundwater ages allow (Datta et al., 2011). This leaves septic systems as a potential source of wastewater and organic carbon.

McArthur et al. (2012) have used Cl/Br ratios to examine the possibility of septic and wastewater infiltrating into shallow aquifers. Septic water was found to be infiltrating and mixing with groundwater due to elevated Cl/Br ratios and the presence of fecal coliform bacteria (McArthur et al., 2012). The mixing was found to be greater in groundwaters present in the paleo-channel facies and minimal in the interfluvial facies. The lack of the clay/silt confining layer allows septic discharge to infiltrate the shallow aquifers and mix with aquifer water. The margins of the interfluvial facies are impacted

by wastewater infiltration due to drawdown effects of pumping for domestic and irrigation use (McArthur et al., 2012).

The influx of organic carbon from waste water could lead to enhanced redox conditions favoring the release of As from Fe-oxyhydroxide minerals (Polizzotto et al., 2005; McArthur et al., 2012). However, McArthur et al. (2012) found low As concentrations where Cl/Br ratios and fecal coliform counts indicated strong mixing with wastewater. This indicates that the wastewater may be reducing As contamination in these areas. Wastewater contains NO_3, which is preferentially reduced over Fe and could slow the release of As from Fe-oxyhydroxide minerals (McArthur et al., 2012). Urine also contains high concentrations of SO_4, which when reduced could trap As in pyrite grains, reducing contaminant concentrations (McArthur et al., 2012). The effects of wastewater on As contamination is not well understood and McArthur et al. (2012) suggest the impact has been underestimated by previous investigators (McArthur et al., 2004, 2001).

4.1 Role of Organic Matter

Fe oxide minerals deposited with organic matter can enhance geochemical reactions leading to the mobilization of As (Saunders et al., 2008). The role of organic matter in the mobilization of As has been a focus of As research in India and Bangladesh. Microbial reduction of organic matter can create reducing conditions that favor the reductive dissolution of As-bearing Fe-oxyhydroxide minerals (McArthur et al., 2001). Buried peat deposits have been thought to be responsible for providing the redox conditions favorable to the release of As from FeOOH minerals (McArthur et al., 2001). Peat deposits are common throughout the Bengal Basin (Ahmed et al., 2004) and TOC values in groundwaters measured at or just above 6% indicate the presence of peat (Nickson et al., 1998). However, the idea of peat being the dominant source of organic matter that contributes to As release from Fe-oxyhydroxides has been challenged by recent investigations (McArthur et al., 2004). Peat deposits were discovered in As-free areas while contaminated areas nearby were free of peat deposits (Pal et al., 2002). This could indicate that there are surface and subsurface sources of organic matter and that they all impact the local distribution of As contamination in aquifers (McArthur et al., 2004).

Needle sampling has allowed researchers to measure the concentration of the particulate and dissolved As phases at specific depths (van Geen et al., 2008). This detailed sampling procedure has informed flushing models and revealed relationships between phases of mobilized As that indicate

that influx of organic matter from surface sources would be offset by increased flushing of aquifer sediments (van Geen et al., 2008).

Characterization of organic matter from shallow aquifers in West Bengal show that some of the organic matter was sourced from terrestrial flora deposited in a fluvial environment, consistent with the sedimentology studies of Holocene sequences (Rowland et al., 2006). However, evidence of organic matter derived from petroleum was also discovered. Petroleum has been identified in the region at depth of about 5.6 km and could have migrated upward where it underwent biodegradation by microbes in shallower environments (Rowland et al., 2006). This finding is specific to this study area and more extensive research needs to be conducted to determine if organic matter derived from deep hydrocarbons is present on other parts of the Bengal Basin aquifers (Rowland et al., 2006).

Farooq et al. (2012) examined the influence of jute processing-derived dissolved organic carbon (DOC) on arsenic enrichment within the Bengal Basin. Jute processing is a widely accepted practice for obtaining jute fibers from their respective plants, and entails rotting the plants in ponds for weeks at a time. This process produces noticeable amounts of DOC and was investigated to determine the role that it plays in As release and accumulation. The results suggested that DOC induced As mobilization was greatly enhanced in the zone at which the percolating DOC-rich pond water infiltrated the most (uppermost ~2.6 m). Henceforth, jute-processing ponds were found to contribute DOC to shallow groundwaters and ultimately enhance As pollution. With time, this pond water may infilter deeper and perhaps enhance deeper aquifer dissolved As concentrations with time.

5. GEOLOGY

Arsenic contamination in India and Bangladesh is mostly restricted to the Bengal Basin and the Ganges Plain. The evolution of the basin, its stratigraphy, and current hydrological regime impact the extent and variability of As contaminations in this area. The Bengal basin is the largest deltaic system in the world and is home to 2% of the world's population (Mukherjee et al., 2009; Ahmed et al., 2004). This delta system drains the Ganges, Brahmaputra, and Meghna Rivers into the Bay of Bengal and the Indian Ocean. These rivers transport about 1 billion tons of sediment per year (Goodbred and Kuehl, 2000a). The huge sediment load discharges into the Bay of Bengal

where it forms a huge, prograding delta termed the Ganges—Brahmaputra—Meghna (GBM) Delta (Ahmed et al., 2004). This delta comprises about 100,000 km^2 and is the largest subaerial delta in the world (Goodbred and Kuehl, 2000a).

The formation of the Bengal and Ganges basin are the result of a continent—continent collision that occurred when the Indian subcontinent collided and subducted beneath the Eurasian and Burmese plates beginning in the mid-Oligocene (Mukherjee et al., 2009; Allison, 1998). Subduction of the Indian plate under the Eurasian plate is north—south, while subduction under the Burmese plate occurs along a north east—southwest trend (Muhkerjee et al., 2009). The Himalaya and Indo-Burmese mountains begin to uplift in the mid-Miocene, which created the Himalayan foredeep (Allison, 1998). The basin is bounded by the Indian shield to the west, the Shillong Plateau to the north, and the Indo-Burman mountains to the east (Muhkerjee et al., 2009; Allison, 1998). This foredeep basin filled with around 15 km of Cenozoic alluvium sediment transported by the Ganges, Brahmaputra, and Mehgna Rivers forms what we now call the Bengal Basin (Morgan and McIntire, 1959; Ahmed et al., 2004 Allison, 1998). Tectonic stress on sediments has created extensive faulting within the basin that has partitioned the Bengal Basin into subbasins (Goodbred and Kuehl, 2000b; Allison, 1998). The tectonic alteration is greater in the north and eastern parts of the basin (Goodbred and Kuehl, 2000b). The development of these subbasins has exerted influence on sedimentation, creating a heterogeneous stratigraphy (Muhkerjee et al., 2009). The Bengal basin developed during glacial and intraglacial time periods and the eustatic sea level changes have also affected the stratigraphy of the basin (Biswas and Roy, 1976). Mukherjee et al. (2009) has described the evolution of the Bengal Basin in terms of geology, geomorphology, and hydrology. Figure 8 provides a comprehensive geological map of the Himalayan foreground and Bengal Basin.

Tectonics and sea-level change have exerted a strong influence on the stratigraphic development of the Bengal basin (Goodbred and Kuehl, 2000b). Interbasin faults have divided the basin into sub basins, complicating the hydrology of the area. Changing sea levels during periods of glaciation has influenced the stratigraphy of the Bengal basin and the spatial distribution of As in groundwater (Hoque et al., 2012). During the last glacial maximum in the Pleistocene from ~125 to 18 ka, sea levels were almost 130 m lower (Goodbred and Kuehl, 2000b). This caused the rivers flowing from the Himalaya Orogeny to cut channels 30—50 m below current elevations (Hoque et al., 2012). The sediment exposed in the interfluvial areas

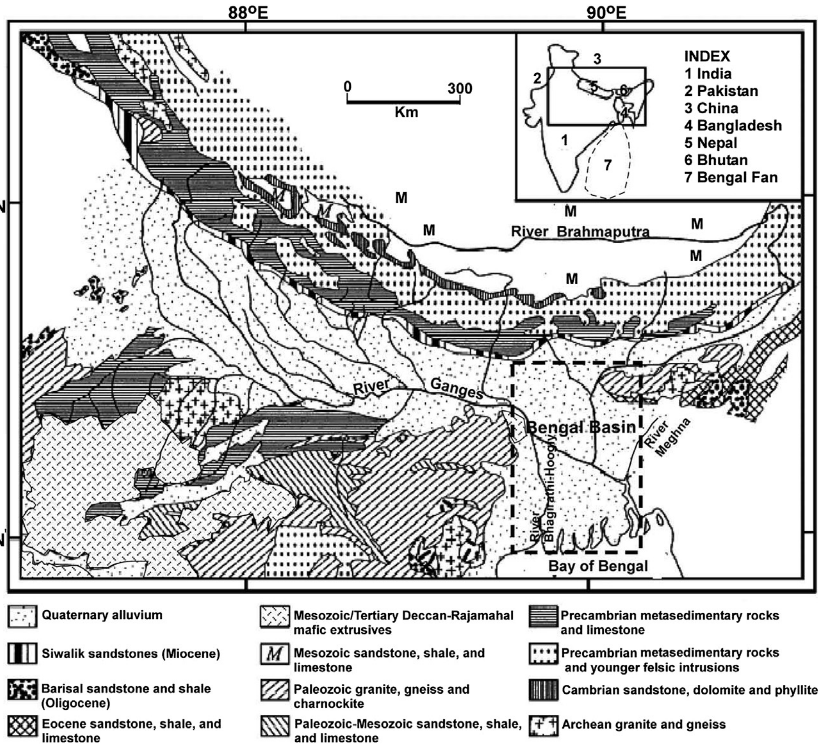

Figure 8 Geological map of Himalayan foreground and Bengal Basin (Mukherjee et al., 2009). *Reprinted from Abhijit Mukherjee, Alan E. Fryar, William A. Thomas, 2009. Geologic, geomorphic and hydrologic framework and evolution of the Bengal basin, India and Bangladesh. J. Asian Earth Sci. 34 (3), 227–244, with permission from Elsevier.*

during the transgression was subject to extensive erosion, and an oxidized red clay paleosol was thereby formed (Hoque et al., 2012). The Last Glacial Maximum paleosol (LGMP) was described by McArthur et al. (2008) and the laterite layer was described by Goodbred and Kuehl (2000a). This paleosol and its lateral extent has important implications in understanding the spatial variability of As in the Bengal basin and will be further discussed later (McArthur et al., 2008; Hoque et al., 2012). After 18 ka, the glaciers began to recede and sea levels rose and the delta flooded. Sediments were deposited in the fluvial channels and formed the gray channel fill sediments observed today (Hoque et al., 2012).

Geomorphic features throughout the Bengal Basin have been shown to correlate to high As areas (Ahmed et al., 2004). The main geomorphic units in the Bengal Basin are tertiary hills, Pleistocene uplands, and Holocene

plains (Ahmed et al., 2004; Ravenscroft et al., 2005). Holocene lithofacies have been identified as either a sandy channel fill or a fine grained over bank sediment (Ahmed et al., 2004). Sedimentary structures such as ripple marks, cross-lamination, and fining upward sequences reveal a fluvial depositional environment for these sediments. These stratigraphic sequences are summarized in Table 3.

The origins of the sediments within the Bengal basin (Himalayan an Indo-Burmese mountain ranges) exert a strong control on the mineralogy found in the basin (Mukherjee et al., 2009). Tertiary sediments form sandstone and shale sequences while the Pleistocene sediments are mainly clays. These clay sequences are overlain by Holocene alluvium (Ahmed et al., 2004). Mineralogical analysis of Holocene sands from Bangladesh have revealed the presence of quartz (47—70%), lithic grains (4—17%), biotite and muscovite (2—15%), feldspar (1—8%), and chlorite (trace-8%) (Ahmed et al., 2004). The sands have been described as immature to sub mature with minimal matrix material (Ahmed et al., 2004). The provenance of the sediments is from recycled orogenic material from the Himalaya and Indo-Burmese mountains (Ahmed et al., 2004).

6. HYDROLOGY

Quaternary sediments are good aquifers throughout the region and are used extensively for drinking and irrigation (Ahmed et al., 2004). As contamination is generally restricted to the Holocene alluvial sediments at shallow depths, which are predominantly composed of sand, silt, and clay (Ahmed et al., 2004). Groundwater can be reached at shallow depths (10—60 m) in major Holocene aquifers and fluctuate seasonally (Ahmed et al., 2004; Ravenscroft et al., 2005). In Bihar, the water table can be reached at around 6.8 m depth during the premonsoon season and fluctuates by 1.57 m during the changing seasons (Saha et al., 2011). Groundwater flows slowly from north to south due to low topographic relief (Ahmed et al., 2004). In hilly areas, Pliocene sands form the major aquifers (Ahmed et al., 2004). Holocene aquifers are mainly unconfined while Pliocene aquifers and deep alluvial aquifers are confined and generally uncontaminated with As (Ahmed et al., 2004). The aquifers of the Bengal Basin are laterally bounded by high topography landforms to the east and west (as described in the geologic setting section), which receive significant amounts of annual rainfall and contribute to the water budget of the Bengal Basin (Michael and Voss, 2009).

Table 3 Generalized stratigraphy of Bengal Basin

Age	Stratigraphic units	Lithology	Notes
Late Pleistocene—Holocene	Chandina formation Dhamrai formation Unclassified deposits	Upward fining, gray micaceous, medium and coarse sand to silt with organic mud and peat.	Forms major aquifers beneath recent floodplains. Probably <150 m thick.
Lower Pleistocene	Madhupur clay Barind clay	Tought, red-brown to gray, silty-clay; residual deposits; kaolinite and iron oxide.	Often absent beneath Holocene floodplain. Thickness 6—60 m.
Plio-Pleistocene	Dupi Tila formation Dihing formation	Yellowish-brown to light grey, medium and coarse sand to clay; very weakly consolidated; depleted in mica and organic matter.	Forms major aquifers beneath the terraces and hills, and deeper aquifers beneath the Holocene floodplains. Hundreds to thousands of meter thick.
Tertiary	Tipam Group	Yellowish-brown, weakly consolidated sandstone and mudstone.	Minor aquifers in hills.
	Surma series	Consolidated sandstone and shale.	No significant aquifers.
	Barail series	Consolidated sandstone and shale.	
	Jaintia group	Shale, limestone and sandstone.	
Mesozoic	Sylhet traps	Basalt, shale and sandstone.	

The aquifer system has historically been described as a two-tier aquifer, with an upper aquitard of clay and silt overlying a composite aquifer, which is tapped by many hand dug-tube wells (Ahmed et al., 2004; Saha et al., 2011). The upper 25 m of the shallow aquifer is composed of sandy-clay

and silty-clay sediments (Saha et al., 2011). Stable isotopic analyses indicate that this upper clay-rich interval does not act as an aquitard but is instead part of the upper portion of the aquifer (Saha et al., 2011). This upper layer has been found to contain black laminae, which is indicative of organic matter deposition (Saha et al., 2011). Below the composite aquifer is the main aquifer of coarse sand and gravel, which is utilized for irrigation and municipal water supply. The lower aquitard separates this main aquifer from the deep aquifer (Ahmed et al., 2004). This description can be simplified by dividing the system into a shallow aquifer (above 200 m) and a deep aquifer (below 200 m) that are separated by a thick clay layer (Ahmed et al., 2004; Saha et al., 2011). The lower aquifer is confined to semiconfined and typically has a higher peizometric head during the premonsoon season (Saha et al., 2011). The aquifer system has not been well defined, and groundwater flow is complicated by large-scale irrigation practices (Mukherjee et al., 2009). In West Bengal, the main aquifer can be found at depth maximum of 50–80 m in the northern part of the state to 180–200 m in the south (Mukherjee et al., 2007). Discontinuous clay layers in the southern portion of West Bengal divide the main aquifer and create small confined aquifers (Mukherjee et al., 2007). Confined aquifers below ~300 m depth can have different chemical composition, indicating different flow regimes (Mukherjee et al., 2007).

As contamination is mainly restricted to the shallow Holocene aquifer throughout the Bengal Basin (Ahmed et al., 2004). Reliable dating of these sediments is not available and so the Holocene aquifer typically refers to the shallow aquifers under Holocene age alluvial, estuarine and deltaic flood plains (Ravenscroft et al., 2005). In West Bengal, the shallow aquifer occurs at a depth of 12–15 m below the surface and is comprised of subrounded to subangular fine to medium-grained sand with many heavy minerals suggesting both volcanic and metamorphic origin (Das et al., 1996). Except at the southern boundary, this aquifer is unconfined and interconnected with deeper aquifers in the area (Das et al., 1996). Unconfined aquifers mainly occur in the districts of Nadia, Murshidibad, and Malda while semiconfining conditions exist further south in Bardhaman and North and South 24-Paraganas (Bhattacharya et al., 1997). Aquifers with depth ranges between 35 to 46 m and 70–150 m also occur throughout eastern West Bengal (Das et al., 1996). The intermediate aquifer contains subrounded to subangular medium sand with heavy minerals that suggest metamorphic provenance (garnet, biotite, kyanite, opaques) and the deeper aquifer contains rounded to subrounded, fine to coarse sand with minerals that suggest a more igneous

provenance (altered biotite, rutile, tourmaline, etc.) (Das et al., 1996). The sediment profile reveals a fining upward sequence that indicates fluvial depositional environments (Bhattacharya et al., 1997). These aquifers are recharged in mountainous fronts and surrounding upland terraces (Bhattacharya et al., 1997). In West Bengal, the intermediate aquifer is typically polluted with As while the deeper aquifer is free of As. The confining layer is laterally discontinuous and connectivity between upper and lower aquifers can lead to As pollution with time (Das et al., 1996; Bhattacharya et al., 1997).

6.1 Aquifer Recharge

Recharge throughout the basin is controlled by the monsoon season (Mukherjee et al., 2009). Around 500 cm of rain can fall in the upland regions surrounding the Bengal Basin (Mukherjee et al., 2009). 82.21% of the annual rainfall usually occurs during the monsoon season (July–September), 16.23% falls in the premonsoon season (January–May) and only 1.57% falls postmonsoon (October–December) in the Bengal Basin (Mukherjee et al., 2009). The heavy monsoon rains recharge the aquifers and raise the water table substantially, sometimes creating flood conditions (Ahmed et al., 2004). As-enriched aquifers have revealed ^{3}H values that indicate modern recharge within the past 50 years (Saha et al., 2011).

Seasonal examination of stable isotopes provides information on aquifer recharge and can help define the recharge sources for groundwater. Mukerjee et al. (2007) examine the stable isotope composition of West Bengal precipitation and groundwater throughout the premonsoon, monsoon, and postmonsoon seasons. Their local meteoric water line (LMWL) fits well with previous research (Krishnamurthy and Bhattacharya, 1991) and data indicate distinct differences between premonsoon and monsoon isotopic concentrations (Mukherjee et al., 2007). Samples collected during the monsoon season were about 5% lighter in $\delta^{18}O$ than premonsoon samples. This indicates premonsoon rains were sources from local surface water bodies that had already undergone evaporative enrichment and monsoon rains were sourced further away (Mukherjee et al., 2007). Signatures of evaporative enrichment of recharged water were also found in Bihar (Saha et al., 2011).

Deep groundwater samples were collected and analyzed for stable isotopes for comparison with precipitation values (Mukherjee et al., 2007). The groundwater samples fell within the range of monsoonal precipitation indicating the deep aquifers were recharged in a similar climate as the

present. Other researchers have found a wider range in stable isotopic data from deep groundwater (Aggarwal et al., 2000). They suggested that the deeper aquifer was recharged during a climate different from the present. Without radiometric age data and with different sample locations, the two studies cannot be properly evaluated (Mukherjee et al., 2007).

Sewage waters, surface water bodies, and irrigation water contribute to the recharge of the shallow aquifer (Saha et al., 2011). These recharging waters are enriched in stable isotopes due to evaporation (Saha et al., 2011).

6.2 Groundwater Flow

Topographic relief is low throughout the Bengal Basin, which creates a low horizontal hydraulic gradient (Michael and Voss, 2009). Low gradients can create hydrologic flow regimes that are localized to certain areas, creating small-scale flow cells in the shallow aquifer (Michael and Voss, 2009). The vertical hydrologic conductivity is variable, which serves to reduce the hydrologic gradient and favors horizontal flow (Michael and Voss, 2009). Tritium measurements of groundwater indicate the shallow water at approximately 100 m depth is less than 100 years old (Aggarwal et al., 2003). Harvey et al. (2005) found groundwater above 60 m to be less than 45 years old. Young groundwater ages indicate rapid recharge of the aquifers, which can lead to mobilization of As (Harvey et al., 2005). The extensive use of groundwater for irrigation creates complications for accurately determining groundwater ages because the irrigation water is being recharged back into the aquifer, mixing older and younger water together (Harvey et al., 2005). If groundwater used for irrigation has an age that precedes atomic testing, tritium values can be minimal, and when mixed with younger water could reduce the tritium concentration, affecting the measured age (Harvey et al., 2005).

Low ^3H values for water in the lower portions of the upper aquifer indicate slow vertical flow which could be due to silty-clay facies that are present throughout the aquifer (Saha et al., 2011). Lower flow rates could increase As mobilization due to prolonged contact between sediments and water (Saha et al., 2011). The upper aquifer shows similar isotopic concentrations between high As and low As depths which shows the upper aquifer is well connected and subject to the same hydrologic flow regime (Saha et al., 2011).

6.3 Effects of Aquifer Pumping

The impact of groundwater pumping on local and regional hydrologic gradients is important to consider. Pumping from deep wells could create flow

conditions favorable to the downward transport of As from contaminated horizons (Michael and Voss, 2009). Pumping during the dry season can easily be greater than recharge and create strong cones of depression drawing water down toward well screens (Charlet et al., 2007). However, it has been suggested that groundwater pumping for irrigation does not have significant impacts on the residence time of groundwater (Harvey et al., 2005). Drawdown initiated by pumping could reduce groundwater discharge into streams and rivers, and could offset the effects of drawdown within the aquifer if the two values are similar (Harvey et al., 2005). Modeling the impacts of groundwater pumping is complicated by the fact that as of 2004 there were over 1 million irrigation wells in Bangladesh and over 250,000 in West Bengal (Michael and Voss, 2009). To compensate for this, modelers have estimated irrigation withdraw based on areal extent (Michael and Voss, 2009). Models indicate that pumping of the aquifer for irrigation becomes the dominant factor controlling groundwater flow in the basin (Michael and Voss, 2009). The hydrologic flow becomes increasingly vertical as pumping continues and draws down surface water, increasing the recharge (Michael and Voss, 2009). The complex variability of geology and hydrological flow conditions throughout the Bengal Basin makes modeling complex and highly dependent on geological conditions (Michael and Voss, 2009).

Connectivity between upper and lower aquifers influences the downward migration of As contaminated groundwater induced by irrigation pumping. Models have suggested that the deep aquifer flow regime could be consistent over a regional scale and is recharged predominantly at the low As highland surrounding the Bengal Basin. If this continues to be the case, the deep aquifer could be safe from pumping-induced contamination (Michael and Voss, 2009). However, there is evidence that the lower aquifer is not completely isolated from shallower contaminated aquifers, which could lead to contamination of the generally low As deep aquifer (Mukherjee et al., 2011).

6.4 Paleo-Geomorphology

Paleo-fluvial and paleo-interfluvial facies exist throughout shallow/intermediate aquifer within the Bengal Basin and influence the lateral distribution of As in groundwater (McArthur et al., 2008). The two facies are delineated by the presence of a red paleosol that developed in the interfluvial environments during the last glacial maximum when sea level was low (McArthur et al., 2008). This is termed as LGMP by McArthur et al. (2008) which

will be used throughout this paper. Rivers traveling to the Bay of Bengal cut through sediment and created channels through Pleistocene sediments where organic matter was deposited (McArthur et al., 2008). When glaciers began to recede and sea levels rose, the river channels were filled with sediment, burying the channels and organic matter (McArthur et al., 2008). The paleochannels are not confined by the paleosol and the presence of organic matter enables redox conditions that support the mobilization of As from sediments into the groundwater (McArthur et al., 2008; 2010). Paleochannels contain Pleistocene and Holocene gray sands, which are reduced in nature and can contain high levels of As. The interfluvial facies contain Pleistocene brown sand under the LGMP with gray silt deposits above the paleosol (McArthur et al., 2008, 2010). These facies are depicted in Figure 9 from McArthur et al. (2010).

The lateral extent of these paleo-channels and interfluvial zones exert influence on As occurrences as the LGMP prevents vertical migration of groundwater and contaminants. However, horizontal migration of groundwater and contaminants can affect the low As interfluvial aquifers (McArthur et al., 2010). Recharge into the unconfined paleochannel aquifer can force

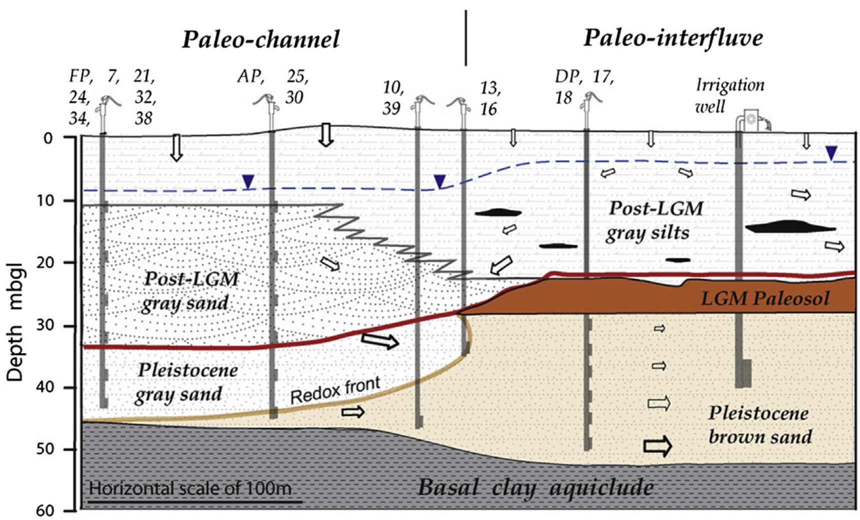

Figure 9 Schematic diagram showing the architecture of paleo-channel and interfluvial sedimentary facies for the shallow aquifers in Bengal Basin (McArthur et al., 2010). *Reprinted from McArthur, D. M., Banerjee, S., Sengupta, P., Ravenscroft, S., Klump, A., Sarkar, B., Disch, R., Kipfer, J. M., 2010. Migration of As, and* $^{3}H/^{3}He$ *ages, in groundwater from West Bengal: implications for monitoring. Water Res. 44 (14), 4171–4185, with permission from Elsevier.*

groundwater to migrate horizontally into the paleo interfluvial aquifer (McArthur et al., 2010). This can change the chemical stratification and move the redox front into the interfluvial sediments. These brown sediments contain FeOOH minerals that have the capacity to adsorb As and can mitigate the effects of As contaminated groundwater flowing into uncontaminated zones (McArthur et al., 2010). Well samples over an 8-year period from within the interfluvial facies showed no increase in As concentrations (McArthur et al., 2010). Along the boundaries between the paleo-interfluve and paleochannels the As concentrations did increase, indicating the lateral movement of water from the reducing conditions present in gray sands of the paleochannel to the more oxidizing conditions of the interfluve (McArthur et al., 2010). Irrigation wells pumping water from within the interfluvial zones have created hydrologic gradients that allow contaminated water from paleochannels to flow into the periphery of the uncontaminated interfluve facies (McArthur et al., 2010). The brown sands adsorb As as it travels through the brown sands, which has prevented the contamination of the interior of the interfluve (McArthur et al., 2010).

Shah (2014) investigated the geomorphological role of As distribution within India's Middle Gangetic Plain. Water from 224 tube wells was analyzed between Mirzapur and Patna towns along the Ganga and Ghaghara Rivers (Shah, 2014). Villages within lowlands proximal to active and abandoned meandering rivers contain recently deposited alluvium (Upper Pleistocene-present) and are exposed to As contaminated water derived from shallow tube wells (Shah, 2014). Aquifers containing newer alluvium are characteristic of organic-rich, gray-black argillaceous sediment (3 ± 1 ka) that contain max As concentrations of 30.91 mg/kg (Shah, 2014). Villages occupying regions with older alluvium (Middle to Upper Pleistocene) at higher elevations are well flushed due to a high hydraulic head and are generally absent of organic matter (Shah, 2014) important for the reductive dissolution of iron oxides and release of As into groundwater. Interfluve highlands contain older alluvium (47 ± 12 ka) characterized by a highly oxidized yellow-brown sediment with calcareous and ferrunginous concretions and contain max As concentrations of 13.73 mg/kg (Shah, 2014). Figure 10 represents a schematic cross-section of As contaminated wells based on which alluvial facies they intrude (Shah, 2014). Shah reports the majority (77%) of tube wells containing As contaminated water are characterized by aquifers between depths of 21—40 m within the recently deposited sediment (2013). Rivers such as Yamuna, Ganga, Gomti, Ghaghara, Gandak, Burhi Gandak, and Kosi transport sediment containing high As

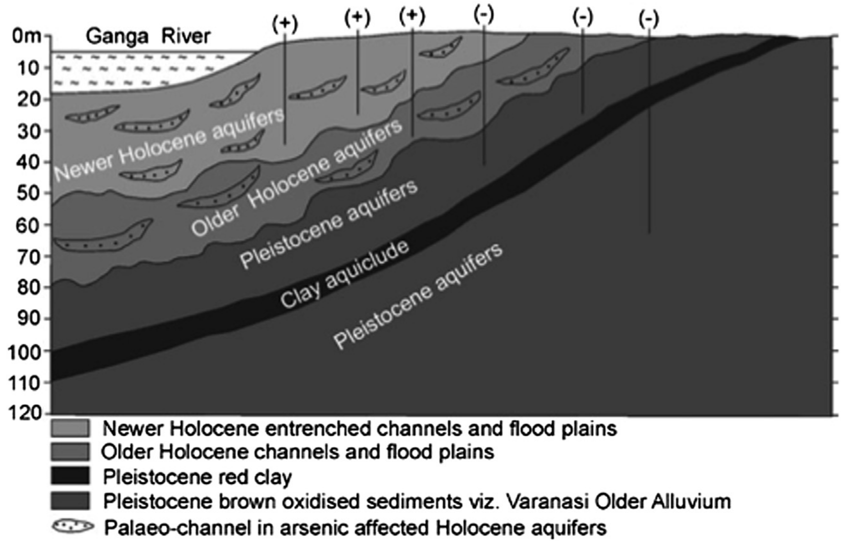

Figure 10 Schematic profile diagram showing As contaminated areas in the Middle Gangetic Plain. Paleo-channels are not shown in the Pleistocene aquifers. Tubewells of As-contaminated and As-safe are shown in symbols (plus sign) and (negative sign), respectively. *Reproduced from Shah (2014). With kind permission from Springer Science and Business Media, Figure 8.*

(10.59 mg/kg max), which may be a significant contributor of As within the region (Shah, 2014).

6.5 Rivers

Several rivers flow through India and the Bengal Basin, a further description of typical As concentrations is further detailed in the Table 4.

Stable isotopes have been analyzed to determine the effect of rivers on groundwater recharge/discharge (Mukherjee et al., 2007). Three rivers were examined (Rivers Ganges–Bhagirathi–Hooghly, River Jalangi, and River Ichamati) and groundwater was sampled in close proximity to the river course (Mukherjee et al., 2007). The River Ganges was found to be more depleted than surrounding groundwater. The river source is located in the Himalayan Mountains and snowmelt contributes to the isotopic depletion (Mukherjee et al., 2007). Throughout the course of the Ganges, the isotopic values do not change significantly, indicating minimal latitudinal effect on the river water (Mukherjee et al., 2007). However, stable isotopes of the groundwater do change with latitude, becoming more enriched toward the south (Mukherjee et al., 2007). Similar isotopic values between

Table 4 Typical As concentrations in the major rivers of India and the Bengal Basin. During the monsoon season, the combined discharge of the Ganges and Brahmaputra Rivers (82,000 m^3 sec^{-1} and 13 $\times$ 10^6 tons per day) is estimated to be transported into the Bay of Bengal

Source	Water ($\mu g\ L^{-1}$)	Riverbed/aquifer sediments ($mg\ kg^{-1}$)	References
Rivers			
Baitrani River	0.1 to 2.1	–	Konhauser et al. (1997)
Mahanadi River	0.1 to 3.0	–	Konhauser et al. (1997)
Ganges River	7.0 to 8.5	2–9	Subramanian et al. (1987), Ramanathan et al. (2005)
Ghagra River	1.9 to 3.4	–	Ramanathan et al. (2005)
Chhoti Saryu River	8.6 to 12.3	–	Ramanathan et al. (2005)
Shivnath River, Chattisgarh	100 to 300		Pandey et al. (2002)
Brahmaputra River	–	2–6	Subramanian et al. (1987)
Yamuna River	–	3–11	Subramanian et al. (1987)
Narmada River	–	3–5	Subramanian et al. (1987)
Tapti River	–	3–5	Subramanian et al. (1987)
Godavari River	–	4–14	Subramanian et al. (1987)
Krishna River	–	2–5	Subramanian et al. (1987)
Cauveri River	–	2–4	Subramanian et al. (1987)
Groundwater			
Groundwater (West Bengal)	<10 to 4600	21–129	Mandal et al. (1996), von Bromssen et al. (2007), Bhattacharya et al. (2002), Sankar et al. (2014)
Groundwater (Bihar)	<10 to >1000	–	Chakraborti et al. (2003), Chakraborti et al. (2004)

Table 4 Typical As concentrations in the major rivers of India and the Bengal Basin. During the monsoon season, the combined discharge of the Ganges and Brahmaputra Rivers (82,000 $m^3\ sec^{-1}$ and 13×10^6 tons per day) is estimated to be transported into the Bay of Bengal—cont'd

Source	Water ($\mu g\ L^{-1}$)	Riverbed/aquifer sediments ($mg\ kg^{-1}$)	References
Groundwater (Jharkhand)	<10 to >50	–	Chakraborti et al. (2003), Chakraborti et al. (2004)
Groundwater (Uttar Pradesh)	<10 to >80	–	Ramanathan et al. (2005), Chakraborti et al. (2003), Chakraborti et al. (2004)
Groundwater (Chattisgarh)		–	Pandey et al. (2002)
Groundwater (Rajasthan)	<1 to 13.8	–	Madhavan and Subramanian (2000)
Groundwater (Tamilnadu)	<10 to 146	–	Ramesh and Ramanathan (2002)*
Lakes			
Lake water	35	–	Konhauser et al. (1997)
Mining areas			
Mine water inlet (Rajasthan)	<1 to 6.9	–	Ramesh and Ramanathan (2002)*
Mine water outlet (Rajasthan)	2 to 140	–	Madhavan and Subramanian (2000)

* Taken from Mukherjee et al., 2005, with permission from CSIRO Publishing http://www.publish.csiro.au/pid/3478.htm.
Adapted from Mukherjee et al. (2005).

the River Ganges and close proximity groundwater in northern latitudes suggests the groundwater is to some degree influenced by recharge from the rivers. As the difference becomes greater to the south, the recharge from the Ganges into groundwater becomes less (Mukherjee et al., 2007). During the dry season, the Hoogly River and surface bodies such as ponds are the main sources of recharge to surrounding groundwater (Charlet et al., 2007).

In the Jalangi River, stable isotopes are more enriched that the surrounding groundwater. This could be due to both evaporation of the runoff and

the narrow stream channel (Mukherjee et al., 2007). There is little stable isotopic change with latitude in the Jalangi River and it does not contribute to groundwater recharge. In fact, the isotopic data and As concentrations measured in the river indicate the groundwater discharge to the Jalangi River (Mukherjee et al., 2007). The Ichamati River and close proximity groundwater show increasing enrichment with decreasing latitude. Isotopic deviations observed near the mouth of the river are due to seawater intrusion and mask river–groundwater interactions (Mukherjee et al., 2007).

7. GROUNDWATER CHEMISTRY

After the discovery of As pollution in India and Bangladesh, extensive research was undertaken to characterize the water chemistry of the affected shallow aquifer. The GMB delta system typically contains anoxic, Ca-Mg-HCO_3 groundwater (McArthur et al., 2001; Mukherjee and Bhattacharya, 2001; Nath et al., 2009). The waters are typically low in dissolved oxygen and nitrates, which have been removed in the reducing conditions of the shallow aquifers (McArthur et al., 2001). pH values are circumneutral to slightly alkaline (Nath et al., 2009; McArthur et al., 2001) and are typically low in Na and Cl except certain areas where marine connate water has persisted in the sediments (McArthur et al., 2001). Groundwater in West Bengal has high bicarbonate values and low Eh values that are not dependent on As values in the groundwater (Nath et al., 2009). As values decrease with depth in West Bengal aquifers, but deeper aquifers are not free of some As pollution (Nath et al., 2009). Groundwaters become more oxidizing with depth, which could be exerting influence on the mobility of As at depth (Nath et al., 2009). Negative correlation between Eh and As concentration with increasing depth supports this notion (Nath et al., 2009).

Further groundwater studies in West Bengal indicate HCO_3^- is the predominant anion and the groundwater has a hardness value of 140 mg L^{-1} equivalent $CaCO_3$ (Charlet et al., 2007). The aquifer temperature ranges between 26° and 28 °C, with slightly higher temperatures recorded near the Hooghly river where recharge is active (Charlet et al., 2007). The average P_{CO_2} value was calculated at $10^{-1.3}$, which is higher than the atmospheric concentration due to the water being in equilibrium with calcite (Charlet et al., 2007). Shallow groundwater is high (4.4 mg L^{-1} average) in DOC due to infiltration of untreated sewage waste (Charlet et al., 2007). Fe (II) concentrations range between 0.010 and 0.017 mmol L^{-1}

and anoxic conditions favor the reductive dissolution of Fe-oxyhydroxides (Charlet et al., 2007). The most extensive Fe reduction and dissolution occur in paleochannel facies (Charlet et al., 2007) subject to higher recharge and infiltration of surface-derived DOC due to the lack of the confining layer (McArthur et al., 2010; Zheng et al., 2004).

Mukherjee et al. (2011) found the groundwaters in West Bengal to be made up of Ca-Mg-HCO_3 and Ca-Na-HCO_3 composition. The reducing groundwaters consist of low SO_4^2 and NO_3, which do not show correlation with As. Absence of SO_4^2 and NO_3 correlation with As indicate pyrite oxidation. High amounts of NH_4^+ and PO_4^3 were present in the groundwater. In reducing waters such as this, NO_3 is commonly converted to NH_4. Most groundwater in the Bengal Delta Plain contains a positive correlation between high Fe and high PO_4^{3-} and high As up to 70 m. The source for high PO_4^{3-} is not well understood. Elevated PO_4^{3-} levels may be a result of phosphorus being released during oxidation of organic matter (Bhattacharya et al., 2002).

In Murshidabad, water chemistry was characterized by water with circumneutral pH values for high As water and low As waters indicating pH values below and above circumneutral pH values. Higher conductivity values (720–860 μS cm^{-1}) were associated with As-enriched waters, whereas low As waters were found below or above the given range. The fluctuation on the low-As waters could be due to flushing from the higher elevation Pleistocene aquifer. DO and oxidative redox potential in sites of high As were higher than would be expected for high As waters, but were still in the upper range of anoxic or suboxic condition for most samples, likely because of rapid partial oxidation during sampling. The low As water values for DO and oxidative redox potential were fitting for overall highly oxidized aquifers (Neal et al., 2010). In decreasing redox conditions, As is most commonly adsorbed on secondary, oxidized Fe- and Mn-phases in the aquifer from which it is mobilized (Stüben et al., 2003). The reason for decreasing redox conditions is controversial and can vary in differing conditions. One notion is that biodegradation of peat deposits buried in the plain are causing the reducing conditions and thus enhancing the dissolution of FeOOH (Ravenscroft et al., 2005). As has been found to accumulate in peat (Shotyk et al., 1996). Bagla and Kaiser (1996) suggest that the increasing area of flooded rice fields gradually sealed the aquifers from O_2.

Water chemistry from high As areas and collected from hand pumped wells and open, dug wells near the Ganges River in Bihar, India were analyzed for major elements and isotopes to determine recharge pathways

(Saha et al., 2011). 12 out of 13 samples from hand-pumped wells had As concentrations higher than 50 μg L^{-1} and the average concentration was 134 μg L^{-1} (Saha et al., 2011). HCO_3^- is the dominant anion in the groundwater and NO_3 and SO_4 concentrations were low. Mg^{2+} followed by Ca^{2+} were the dominant cations and the pH was slightly acidic (6.86) (Saha et al., 2011). In the shallower, open wells the mineral content of the water was higher, as open wells are more susceptible to infiltration by contaminants (Saha et al., 2011). As concentrations were below the detection limit in all the open well samples (Saha et al., 2011). Cl and SO_4 concentrations were higher in open wells, indicating possible contamination by sewage wastes (Saha et al., 2011). Piper diagrams from deep and shallow wells from Bihar show the chemistry is dominated by HCO_3^- and alkali earth elements while the shallow open wells have significant higher Cl and SO_4 concentrations (Saha et al., 2011). Higher Cl and SO_4 concentrations in hand-pumped wells could indicate infiltration of surface waters containing human and animal wastes (Saha et al., 2011).

The hydrogeochemistry of the deeper aquifer was examined in Bihar and distinct differences were found between the two aquifers (Saha et al., 2011). The lower aquifer typically contains Ca-HCO_3^- facies while the upper aquifer usually contains Mg-HCO_3^- facies, which could be due to the formation of caliche deposits in the upper aquifer (Saha et al., 2011). NO_3 concentrations are lower in the deeper aquifer than the shallower aquifer and As concentrations are generally lower in the deep aquifer (Saha et al., 2011). Little to no ^3H was detected in the lower aquifer, which indicate paleo-recharge and little to no connectivity between the upper and lower aquifers (Saha et al., 2011; Aggarwal et al., 2003). ^{14}C values confirm this paleo-recharge and provide an age of 3 ka to the deeper groundwater (Saha et al., 2011).

Groundwater chemistry in Uttar Pradesh has been characterized to discover any chemical associations with As. Similarly to West Bengal and Bangladesh, groundwater in Uttar Pradesh is only slightly alkaline and has a negative oxidation-reduction potential (ORP) (Chauhan et al., 2009). Nitrate concentrations are below 1 mg L^{-1} despite being in a heavily agricultural area. This could indicate fast recharge and flushing of the aquifer or microbial metabolism (Chauhan et al., 2009). Total Fe values range from less than 1−13.2 mg L^{-1} and is thought to be mainly ferrous Fe due to the negative ORP values in the aquifer. Sulfate values are low, less than 50 mg L^{-1} Chauhan et al. (2009) found wherever As exceeded 50 μg L^{-1}, sulfate was less than 10 mg L^{-1}. This indicates that As release

is not associated with sulfide minerals. As concentrations in the Ballia district of Uttar Pradesh were measured from 56 wells and only 5 were within the WHO limit of 10 μg L^{-1}. 61% of the samples were below 50 μg L^{-1} and the maximum concentration was 468 μg L^{-1} (Chauhan et al., 2009). The deeper aquifer was also tested for As and all groundwater samples exceeded 10 μg L^{-1} but were less than 20 μg L^{-1} (Chauhan et al., 2009).

The nature of the microbial processes in the sediment play a major role in the redox processes that lead to As mobilization. Microbial processes result in transformation of Fe(III) as well as As(V) to As(III), which results in mobilized As in the groundwater. Bacterial activity is increased in recent sediment deposits with high organic matter, and it is proposed that this causes accelerated diagenetic processes and mobilized As (Mukerjee et al., 2011). Bacterial reduction of Fe(III) to Fe(II) leads to a conversion of As(V) to As(III) and may mobilize As (Bhattacharya et al., 2002). In deeper groundwater, reductive dissolution of Fe and Mn oxyhydroxides leads to the mobilization of As (Biswas et al.,. 2011).

Biswas et al. (2014a) investigated the effect of the competing ions in mobilization of arsenicusing surface complexation modeling of the temporal variability of As as well as its speciation in groundwater based on a continuous monitoring of groundwater chemistry in piezometric wells over a period of 20 months. The results suggested that PO_4^{3-} is the greatest competitor, followed by ferrous iron, silicic acid, and then bicarbonates. The study also indicated that a small change in pH has significant effect on the concentrations of dissolved As(III) and As(V) in groundwaters. The overarching conclusion from this study was that As release into groundwater is a complex process involving microbially mediated reductive dissolution of Fe-oxyhydroxide minerals and other similar redox-driven processes.

Distribution of As in groundwater has been found to be laterally patchy or heterogeneous, with areas of low arsenic tens of meters away from high arsenic "hot spots." The cause of the patchy distribution of As has important implications for the mobilization of As as well as determining safe areas to extract safe groundwater. Trends in groundwater chemistry observed in these hot spots can suggest which mechanism of mobilization is prevalent in the aquifer system (Charlet et al., 2007). One such hot spot was examined by Charlet et al. (2007) in West Bengal, India. In this hot spot, As was found sorbed to Fe-oxyhydroxide minerals as well as being present in carbonate and mica minerals (Charlet et al., 2007). Recharging waters are high in DOC and contribute to reducing conditions allowing for the dissolution of Fe-oxyhydroxide minerals. This can lead to an increase in P_{CO2}

concentrations, inducing carbonate dissolution (Charlet et al., 2007). Recharge of DOC-rich surface waters is highest in paleo-channel/paleo-meander geomorphic settings and creates the conditions necessary for the reductive dissolution of Fe oxides and the release of As from sediments (Charlet et al., 2007). Additionally, Farooq et al. (2012) experimentally proved the presence of DOC as mobilizing As from sediments to groundwater, and McArthur et al. (2010) observed As hot spots occurring in the paleo-channel geomorphic features. Horizontal groundwater flow through the Bengal Basin is slow, but irrigation during the postmonsoon season can change the flow patterns from horizontal dominated to vertical dominated which can cause As hot spot plumes to migrate toward wells utilized for irrigation and drinking (Charlet et al., 2007). As hot spots appear to be affected by paleo-geomorphic features, anthropogenic influences of irrigation pumping, and infiltration of anthropogenic DOC from the surface (Charlet et al., 2007).

Groundwater chemistry has also been found to change in proximity to recharge site, such as rivers and ponds, during the postmonsoon season (Charlet et al., 2007). In the Hooghly River in West Bengal, pH values are around 8.25 while the groundwater pH decreases to 6.9 within 1 km (Charlet et al., 2007). The alkalinity of the Hooghly was measured at 6.1 mmol L^{-1} and increases in the groundwater between 15 and 22 mmol L^{-1} (Charlet et al., 2007). Increases in groundwater pumping during the postmonsoon season can lead to increased drawdown and recharge from surface water bodies (Charlet et al., 2007). This could increase the pathways for surface-derived organic carbon to infiltrate the aquifer and contribute to geochemically reducing conditions.

7.1 Seasonal/Temporal Variation

The climate of the Bengal Basin can be described in three broad seasons. The winter season is mild and occurs from October to March and receives the least rainfall. The summer season is hot and occurs from March to June. The monsoon seaon occurs from May/June to October and is characterized by heavy rainfall and warm temperatures (Bhattacharya et al., 2011). The peak rainfall occurs during the monsoon season between June and September with 500 to 4000 mm precipitating into the basin (Michael and Voss, 2009; Bhattacharya et al., 2011). The highest amount of rain falls on the hilly regions that border the basin (Michael and Voss, 2009).

Arsenic occurrences in groundwater from seasonal variation appear to be caused predominantly by dilution differences during monsoon and

postmonsoon (Farooq et al., 2011). The pH measured pre- and postmonsoon in Uttar Pradesh were found to be within 0.01 (Kumar et al., 2010a). Alkaline pH values could be due to dissolution reactions with soil sediments, which create slightly alkaline conditions (pH = 7.8) (Kumar et al., 2010a). HCO_3^- values were higher in postmonsoon samples and could be due to the flushing of CO_2-rich water formed from the decomposition of organic matter (Kumar et al., 2010a). Reduced levels of HCO_3^- in premonsoon samples could be due to carbonate precipitation (Kumar et al., 2010a). Cl^- concentrations were lower in the postmonsoon season than in the premonsoon season and could be due to recharge flushing of the aquifer during the monsoon season (Kumar et al., 2010a). High Cl^- values found in the premonsoon season could be due to septic effluent entering the aquifer, changing evaporation rates during recharge or sediment interactions (Kumar et al., 2010a). Ca^{2+} and Mg^{2+} increased in the postmonsoon samples indicating the dissolution of carbonate minerals and consistency with increases in HCO_3^- values (Kumar et al., 2010a). Seasonal variation of cations and anions was also reported for the Murshidabad district of West Bengal (Farooq et al., 2011). The cations and anions all decreased from premonsoon to monsoon to postmonsoon probably due to dilution by the rainwater. Unlike the Kumar et al. (2010a) study, Ca^{2+} and Mg^{2+} decreased throughout the seasons and the data plotted on a piper diagram show no significant changes in the groundwater type (Farooq et al., 2011). As concentrations from districts in Uttar Pradesh slightly decreased from the premonsoon to postmonsoon samples (Kumar et al., 2010a). In Bangladesh a slight change (10–16%) was seen throughout the premonsoon, monsoon, and postmonsoon seasons (Tareq et al., 2003).

Seasonal changes in As concentrations in groundwater could help explain release mechanisms and provide information for water management practices. Saturated conditions during the monsoon season serve to dilute As concentrations and can mobilize As to other wells (Tareq et al., 2003). The Murshidabad district of West Bengal, India showed a slight but noticeable decrease in As concentrations from premonsoon to postmonsoon (Farooq et al., 2011). 14% of wells tested became contaminated (As >50 $\mu g\ L^{-1}$) due to seasonal variability and wells with As concentrations above 50 $\mu g\ L^{-1}$ showed more variability than those with less than 50 $\mu g\ L^{-1}$ (Farooq et al., 2011). In contrast to these changes, no seasonal variation in As concentrations was found in Uttar Pradesh by Chauhan et al. (2009), though sampling in this study occurred over only 1 year. A 3-year study in Bangladesh also found no seasonal variation of As concentrations (Cheng et al., 2005).

8. ACCESSING SAFE DRINKING WATER

Providing water resources that are free of As contamination is needed in this area. There are two ways to provide water resources that are free of As contamination: identifying As-free groundwater and installing water treatment facilities. Various treatment methods have been introduced with limited success.

8.1 Treatment/Remediation Techniques

Two types of techniques, physicochemical and biological, are commonly utilized to create As removal/treatment apparatuses. Examples of physicochemical techniques are adsorption, ion exchange, precipitation-coagulation, membrane filtration, and permeable reactive methods (Mondal et al., 2006). Biological techniques consist primarily of phytoremediation and microbial/biofiltration. It is important to consider cost, availability, necessities to function, and efficiency when choosing a technique, especially in undeveloped rural areas like much of the As contaminated regions in eastern India and Bangladesh. For this reason, remediation is often difficult and thereby not attempted as often as is necessary given the extent and calamity of As pollution worldwide.

Although many successful laboratory tests (Maheswari and Murugesan, 2009; Mondal et al., 2006; Teclu et al., 2008; Beaulieu and Ramirez, 2013) have proven to remove As, such success in the field is extremely rare. Houssain et al. (2005) examined arsenic removal plant (ARP) efficiency by evaluating 577 ARPs in five districts of West Bengal, India. The average price of each plant was roughly $1500 US dollars, which is considerably above what India can afford, especially with 475 (82%) of these plants being deemed "not useful." Sarker et al. (2012) utilized a fixed bed HFO adsorption filtering process in Habra, West Bengal, as well as a hybrid anion exchange resin removal unit at Ashoknagar, West Bengal. Data showed positive results, with treated water remaining $<50\ \mu g\ L^{-1}$ (raw water $\approx$ 140 and 90 $\mu g\ L^{-1}$, respectively) with up to 17,000 (Habra) and 30,000 (Ashoknagar) bed volumes of raw water, over a span of roughly 5 years each (Sarker et al., 2012). More conventional methods that are locally accessible within developing regions like As contaminated India are the safest means of approaching this issue until advanced methods are made in a more economically feasible manner.

8.2 Alternative Water Resources

Identifying areas that will produce high or low As concentrations is important in providing safe drinking water to local peoples. High As concentrations tend to be found in areas with shallow water tables and low topographic relief (Hoque et al., 2011). Several environments within the Bengal Basin have been identified that commonly have low As concentrations. These can occur at shallow or deep depths and are thought to be influenced by a combination of geochemistry, hydrological flow, presence of organic carbon and sedimentation (Hoque et al., 2011). Three generalized environments present throughout the Bengal aquifer system have been identified as having low As concentrations: Holocene floodplains with shallow aquifers, groundwater occurring in oxidized red-bed deposits and deep groundwater (Hoque et al., 2011). The detailed site identification is described in Hoque et al. (2011) and will be summarized here.

Shallow aquifers with low As groundwaters are characterized by a surface soil with >50% loamy silt, shallow water table between 1 and 7 m deep and silt—clay layers less than 15 m thick (Hoque et al., 2011). The low As occurrences in shallow aquifers is restricted above 30 m. Below this depth, the effect of recent recharge is diminished and As is not flushed from the aquifer (Hoque et al., 2011). Red bed occurrences near Plio-Pleistocene sediments that form the Madhupur and Barind tracts (higher topography than the rest of the interior Bengal Basin) contain low As groundwater. These deposits are common throughout the Bengal Basin, however they are discontinuous and occur at different depths (Hoque et al., 2011). Sediment coloration was also found to be indicative of As content (von Bromssen et al., 2007). Black sediments typically promote high As content, whereas white, off-white, and red sediments are understood to contain low As.

Deep aquifers have received attention as having potential to produce As-free drinking water to inhabitants of the Bengal Basin (Hoque et al., 2011; Radloff et al., 2011; Mukherjee et al., 2011; Burgess et al., 2010; Michael and Voss, 2008). The deep aquifers typically occur below 150 m, but are encountered at deeper depths (around 300 m) closer to the coast (Hoque et al., 2011). The deep and shallow aquifers are separated by a clay—silt layer (Burgess et al., 2010). Deep groundwater wells near the coast can contain high salinity values from seawater intrusion and are not suitable for drinking (Hoque et al., 2011; Burgess et al., 2010). A minimal number of the ~75,000 wells that tap the deep aquifer contain undrinkable water due to salinity values (Hoque et al., 2011). Hydrologic modeling and ^{14}C

dating show that shallow and deeper aquifers are parts of different flow regimes (Hoque et al., 2012). The shallow aquifer has a circulation time of less than hundreds of years while the deeper aquifer circulates over thousands of years (Hoque et al., 2012). Recharge in the shallow aquifer as indicated by ^{14}C measurements reveal modern recharge while deeper aquifers have ages of 1000 years before the present (Hoque et al., 2012). Deep aquifer ^{18}O and ^{2}H isotopes do not show a strong evaporative signature, indicating that the deeper aquifer is recharged along the margin of the Bengal Basin, where sediments are thinner and evaporation is less (Hoque et al., 2012).

Deep aquifers in Bangladesh have low concentrations of As compared to the shallower aquifers and are being utilized for drinking and irrigation (Hoque et al., 2011). However, the prevalence of low As water in the deep aquifer does not occur everywhere. Deep aquifers in the western margin of the Bengal Basin are susceptible to As enrichment and are not safe as a drinking water source (Mukherjee et al., 2011). In the western portion of the Bengal Basin, particularly West Bengal, India, the deep aquifer is not completely isolated from the contaminated upper aquifer (Mukherjee et al., 2011). The discontinuous nature of the clay—silt aquitards creates hydrologic anisotropy, which complicates models and could serve to prevent or retard downward migrating contaminants (Michael ad Voss, 2009; Hoque et al., 2012). However, hydraulic connectivity between aquifers means that aquifers considered safe now could become contaminated over time as the effects of pumping draws down As-enriched water from above (Mukherjee et al., 2011; Burgess et al., 2010; Radloff et al., 2011).

Most deep wells drilled to provide safe drinking water have not been monitored over time (Radloff et al., 2011). Recent examinations of deep well As concentrations have shown that around 25% could be contaminated and 14—18% in Bangladesh could contain As >10 μg L^{-1} (Burgess et al., 2010; Radloff et al., 2011). However, an 8-year-study of the water chemistry of the deep aquifer in West Bengal showed no substantial change in the As concentration (McArthur et al., 2010). If the deep aquifers of the Bengal Basin are to be utilized as an As safe alternative to shallow aquifers, the continued viability of the aquifer must be protected (Michael and Voss, 2008). Hydrologic models show that natural flow patterns would not affect deep aquifers as flow is slow and 60% of the recharge zone is not in As contaminated areas (Michael and Voss, 2008). However, pumping water for agricultural irrigation from the deep aquifer could induce a strong hydrologic gradient that would exert a strong downward flow from shallow

aquifers (Burgess et al., 2010). Domestic water use of deeper aquifer resources will also have an effect on hydrological gradient, but not as strong as irrigation (Burgess et al., 2010). A suggested solution for this is to utilize the shallow aquifer for irrigation and reserve the deep aquifer for domestic use. This has been shown to protect the deep aquifer from As contamination by hydrologic models and could provide As-safe drinking water for 1000 years (Burgess et al., 2010; Michael and Voss, 2008). Using As contaminated water for irrigation poses another risk because of the ability of rice to bioaccumulate As (Burgess et al., 2010).

Geochemical reactions occurring as Arsenic flows into the deep aquifer also need to be examined to have a complete picture of how the deeper aquifer could be affected. As arsenic contaminated water flows into deeper aquifers, geochemical changes, such as oxidizing conditions, can trap As (Burgess et al., 2010). Sediments with a high oxidizing capacity, such as FeOOH minerals, could adsorb As and remove it from the groundwater before it is pumped and consumed (Burgess et al., 2010). The geochemical removal of As will be reduced if the sediments have already been exposed to reducing conditions and they have a diminished oxidizing capacity (Burgess et al., 2010). Characterizing the oxidation potential of the sediments is an important step in determining the ability of deep aquifer sediments to naturally reduce As contamination.

Biswas et al. (2014b) examined the hydrostratigraphy within Nadia district, West Bengal, India to better understand the evolution and sustainability of the subsurface hydrology. Two different aquifers (gray sand and black sand aquifers) were revealed and by investigating their hydrgeochemical and sedimentological properties, it was determined that the black sand aquifer can be targeted as a source for As-safe drinking water, albeit it still contains elevated Mn concentrations; therefore, it should only be utilized in times of great necessity. It is also mentioned that the WHO guideline for Mn should be reexamined.

8.3 Rice and Irrigation

As groundwater contamination has been extensively studied and is well understood by the scientific community. However, a recent focus on irrigation practices and its impacts on As in rice and other crops has become a significant issue. Increased As concentrations in rice and soils have been found to be a direct result of irrigating farmland with contaminated groundwater. Such spreading allows for people living outside of the As-affected region to be exposed to the health effects associated with As poisoning, as a number

of crops are consumed elsewhere; rice is particularly dangerous because it is the staple food of south Asia.

During the dry season, farmers choose to irrigate their land because it is inexpensive and provides 55–80% higher grain yields than the rainy season (Sarkar et al., 2012). 60% of this irrigated water comes from aquifers. Agricultural areas with high As groundwater were found to have high As-enriched soil and rice (Roychowdhury et al., 2002), and agricultural land almost always had greater As content than fallow. Duxbury et al. (2003) found that As content in rice was 1.5 times greater in the dry season compared to the summer, correlating directly with excessive irrigation practices. Most As is taken up in the roots of rice plants, rather than the grains themselves, and concentrations up to 20 times higher than those of plants irrigated by uncontaminated water have been reported (Norra et al., 2005).

In terms of health effects and human consumption, the WHO food hygiene concentration limit of As is 1.0 mg kg^{-1}. Rice samples seldom exceed this limit, however a few select cases (Abedin et al., 2002; Rahman et al., 2003) exhibit levels well above. That being said, irrigated regions with contaminated groundwater need to be continually monitored, especially with a number of sites increasing in toxicity with time (Bhattacharya et al., 2010).

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CHAPTER FOUR

Soil Spectroscopy: An Alternative to Wet Chemistry for Soil Monitoring

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Contents

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Abstract

The soil science community is facing a growing demand of regional, continental, and worldwide databases in order to monitor the status of the soil. However, the availability of such data is very scarce. Cost-effective tools to measure soil properties for large areas (e.g., Europe) are required. Soil spectroscopy has shown to be a fast, cost-effective, environmental-friendly, nondestructive, reproducible, and repeatable analytical technique. The main aim of this paper is to describe the *state of the art* of soil spectroscopy as well as its potential to facilitating soil monitoring. The factors constraining the application of soil spectroscopy as an alternative to traditional laboratory analyses, together with the limits of the technique, are addressed. The paper also highlights that the widespread use of spectroscopy to monitor the status of the soil should be encouraged by (1) the creation of a standard for the collection of laboratory soil spectra, to promote the sharing of spectral libraries, and (2) the scanning of existing soil archives, reducing the need for costly sampling campaigns. Finally, routine soil analysis using soil spectroscopy would be beneficial for the end users by a reduction in analytical costs, and an increased comparability of results between laboratories. This ambitious project will materialize only through (1) the establishment of local and regional partnerships among existent institutions able to generate the necessary technical competence, and (2) the support of international organizations. The Food and Agriculture Organization (FAO) of United Nations and the Joint Research Centre of the European Commission are well placed to promote the use of laboratory and field spectrometers for monitoring the state of soils.

1. INTRODUCTION

The Millennium Ecosystem Assessment (2005) stated that soil provides a multitude of land-based ecosystems goods and services supporting and regulating life on the planet. Thus the preservation and sustainable

management of soils is crucial to tackle the main challenges humanity is facing such as food security, climate change, environmental degradation, water scarcity, and biodiversity (Sanchez et al., 2009). Assessing the state of the soil requires complex analytical approaches with high number of parameters at a large number of sites. Unfortunately, consistency of the approaches and the analytical methods among soil surveyors is often lacking (Sanchez et al., 2009; Louis et al., 2014), hindering the exchange of quantitative data with other disciplines, and the implementation of policies for mitigating the main soil threats (e.g., European Commission, 2006). Hence, the growing demand for high-resolution soil data to cover large areas is difficult to be met, due to the lack of such data (Grunwald et al., 2011; Ben-Dor and Banin, 1995). The consistent monitoring of soil health at continental scale depends on the development and implementation of in situ, low-cost, and fast analytical methods, provided that the results obtained by different operators are comparable and that they can be stored in accessible databases, including the standards and raw data for eventual reinterpretation. Over the past 30 years, soil visible, near-, and mid-infrared (Vis-NIR-MIR) reflectance spectroscopy has proved to be a fast, cost-effective, environmental-friendly, nondestructive, reproducible, and repeatable analytical technique (Viscarra Rossel et al., 2006; Soriano-Disla et al., 2014). The technique is mainly used in the laboratory, but its application in situ (Viscarra Rossel et al., 2009), as well as from air- and space-borne sensors, is growing (Ben Dor et al., 2009). As there is large body of evidence showing that spectroscopy can be used to estimate important soil properties, it is time to ask whether this technique is mature enough for routine soil analysis.

- Can the errors associated with spectroscopic predictions be reduced by joining spectral databases, increasing calibration diversity and density, developing focused calibrations for each type of soil or region, or using local samples to augment calibrations for individual fields?
- Are the larger errors associated with soil spectroscopy mitigated by the larger number of samples possible to analyze with this technique?
- What is required to turn soil spectroscopy into an operational technique for soil assessment at field, country, and continental scales?

The main aims of this paper are to (1) describe the actual status of soil spectroscopy, and (2) propose actions to encourage the application of this technique as an alternative to traditional laboratory analyses.

2. VISIBLE AND INFRARED SPECTROSCOPY

2.1 The Visible

Visible (Vis) light covers only a small portion of the electromagnetic radiation being located in the wavelength range between 0.4 and 0.7 μm. Interaction of visible radiation and soil produces energy transition in atoms mainly because of electron processes known as crystal field effect and charge transfer. A scattering effect is taking place as well across the Vis region. Normally, in soils, the electron processes yield broad absorption bands that control the soil color in the visible wavelengths whereas the scattering effects change the albedo sequence of the spectrum base line. Although the spectral response in the Vis region is smear, it is possible to derive quantitative information from the spectral information in addition to the qualitative information as seen by the naked eyes. Owen (2000) wrote an extended review about origin, principle and applications of the UV-Vis spectroscopy.

2.2 The Infrared

Infrared (IR) radiation covers the range of the electromagnetic spectrum between 0.7 μm and 1 mm. Vibrational energy transitions in molecules typically require energy of a frequency that corresponds to the IR region of the electromagnetic spectrum. Hence IR radiation will activate molecular interatomic vibrations, and this provides the basis of the IR spectroscopy technique. An IR spectrum essentially gives a chemical profile of the sample. Electromagnetic radiation consists of electric and magnetic components, but it is the electric vector of IR radiation that interacts with the molecular interatomic bonds to activate different vibrations, resulting in absorption of the IR radiation. There are many types of molecular vibrations that occur on absorption of IR radiation including stretching, bending, and wagging of the constituent atoms in the molecule. For a compound to be IR active, it is required to have covalent bonding. In addition, there must be an oscillating electric field produced during the chemical bond vibrations of the atoms in the molecule (net change in dipole moment). Absorptions of IR radiations are due to vibrations of molecular bonds such as O–H, C–H, N–H, C=O, C–N, N–O, or C=C. Stretching of some symmetric bonds in which the electrons are equally shared will not give rise to IR-active vibrations, e.g., stretching in N_2 molecules (no net change in dipole moment during its chemical bond vibrations). There are numerous textbooks on the principles and interpretation of IR spectroscopy, including those by Herzberg (1945) and

Alpert et al. (1970). IR radiation is divided in three main regions: the near-infrared (NIR: 0.70−2.5 μm), mid-infrared (MIR: 2.5−25 μm) and the far-infrared (FIR: 25−1000 μm[1]). The NIR region (mostly between 1−2.5 μm) shows overtones and combination modes of the fundamental atoms vibrations in molecules that are active in the MIR and FIR regions. The full description of basics, history, and functions of IR spectroscopy can be found in Stuart (2004) and Aenugu et al. (2011). NIR spectroscopy finds applications in several fields such as food science, semiconductor electronics, pharmaceutics, substances identification, and analysis of soil properties (Davis, 1998). Absorptions in MIR spectra can be interpreted in terms of the fundamental vibrations of functional groups present in a sample as the MIR is dominated by fundamental atoms vibrations mostly in bending, stretching, and rotation vibrational modes. Recently the MIR spectral region plays also important role as the NIR spectral region for soil applications.

3. SOIL VIS AND IR SPECTROSCOPY

3.1 Background

Soil spectroscopy is about the identification and analysis of the interaction of Vis-IR wavelengths with soil properties. The main chemical components in soils that interact with electromagnetic radiation across the Vis-NIR, termed also "chromophores," are OH in free water and clay mineral lattice, organic matter, and nonclay minerals, such as iron oxides, carbonates, and salts (Ben Dor et al., 1999). In the MIR, more information on soils is available, such as Si-bearing minerals (mainly quartz) and other fundamental vibrations groups without overtones in the NIR (Janik and Skjemstad, 1995). In general, the MIR region provides better resolved and more intense peaks than the NIR because fundamental vibrations are taking place in this region (Soriano-Disla et al., 2014).

Soil reflectance across the NIR is characterized by well-defined absorption features associated to overtones of O−H and H−O−H stretch vibrations of free water (1.455 and 1.915 μm) and overtones and combinations of O−H stretch and metal-OH bends in the clay lattice (1.415 and

[1] In the remote sensing community, the shorter infrared wavelengths (0.7−1 μm) are termed near-Infrared (NIR), while the portion of the electromagnetic spectrum between 1 and 2.5 μm is named shortwave infrared (SWIR). At lower frequencies, the spectrum is divided into mid-wave infrared (MWIR) between 3 and 5 μm, low-wave infrared (LWIR) between 8 and 14 μm, and far-wave infrared (FWIR) between 14 and 1000 μm.

2.207 μm). A large absorption centered on 0.665 μm can be attributed to organic matter (Ben Dor et al., 1997). Generally speaking, soil reflectance decreases with organic matter (Ben Dor et al., 1997) and water content (Whiting et al., 2004). Absorptions around 0.4, 0.45, 0.51, 0.55, 0.7, 0.87, and 1 μm are characteristics of the presence of ferrous and ferric iron oxides, and are due to the electronic transitions of the iron cations (Ben Dor et al., 1999). Tables of absorption positions and their related soil components are published in Ben Dor et al. (2002), Viscarra Rossel and Behrens (2010), and Soriano-Disla et al. (2014). In addition to chemical soil components, physical soil properties, such as particle size distribution and aggregate size and density, may lead to changes in baseline height, and absorption intensities of the spectral curves through the phenomenon of light scattering or reflection. Ben Dor et al. (1999) extensively reviewed the fundamentals of soil spectroscopy in the Vis-NIR.

In the MIR, due to strong vibration fundamentals, soil spectra are characterized by clearly identifiable peaks linked to organic or mineral compounds. The first two peaks around (3800–3600 cm^{-1}) are associated to O–H stretching in clay minerals (Calderon et al., 2011). They are followed by two less pronounced peaks (3000–2800 cm^{-1}) linked to the aliphatic C–H stretching (Zimmermann et al., 2007). Between 2000 and 1790 cm^{-1}, three successive peaks indicate the presence of quartz (Calderon et al., 2011). The peaks around 1600–1500 cm^{-1} and 1450–1400 cm^{-1} are mainly associated to aromatic and aliphatic compounds, respectively (Bornemann et al., 2010; Demyan et al., 2012; Tivet et al., 2013). Below 1000 cm^{-1}, the interpretation of the different peaks is more difficult because the spectrum results from a mixture of minerals and organic compounds (Calderon et al., 2011; McCarty et al., 2002).

3.2 Spectroscopy for Soil Property Prediction

The use of spectroscopy for soil analyses is fast, cost-efficient, and nondestructive. Furthermore, there is no need for chemical reagents, and it requires minimal sample preparation (Malley et al., 2004; Viscarra Rossel et al., 2006). A single spectrum may contain comprehensive information on various soil components, and can be used to predict these simultaneously (Islam et al., 2003). The rapid development of portable and handheld spectrometers allows using the technique on-site. Bowers and Hanks (1965) were among the first to investigate the spectral characteristics of soil samples by showing correlation between soil spectra and soil moisture. Later, Stoner and Baumgardner (1981) collected a spectral library containing representative

soil samples of the United States, and identified five typical spectral curves corresponding to five soil classes. Early studies, such as those of Dalal and Henry (1986) and Ben Dor and Banin (1995), started to produce soil property predictions based on spectral data and multivariate statistics. Since then, most of the studies adopted the same quantitative approach and spectroscopy has been exploited to predict soil properties such as organic carbon (Gobrecht et al., 2014), texture (Sørensen and Dalsgaard, 2005), cationic exchange capacity (CEC) (Cañasveras Sánchez et al., 2012), total phosphorus (P) (Abdi et al., 2012), exchangeable potassium (K) (He et al., 2005), and electrical conductivity (Ben Dor et al., 2002; Viscarra Rossel et al., 2006; Todorova et al., 2011). Soriano-Disla et al. (2014) extensively reviewed soil spectroscopic models published in the literature, and listed soil properties that could be determined by means of diffuse reflectance spectroscopy: soil water content, clay, sand, soil organic carbon (SOC), cationic exchange capacity (CEC), exchangeable Ca and Mg, total N, pH, and total concentration of potential pollutant metals/metalloids (As, Cd, Hg, and Pb). Moreover, the indirect interaction between the chromophores and other soil properties can provide additional quantitative information about some soil properties with no active chromophores (Ben Dor et al., 1999). Obviously, soil properties that are directly related to the chromophores (organic carbon, carbonates, etc.) are generally better predicted than the other properties for which the prediction mechanism relies on their correlation with the chromophores within a given spectral library (Reeves et al., 2006). The prediction of soil properties based on spectroscopy registered a tremendous increase in the last decades (Bellon-Maurel and McBratney, 2011). Due to the minor sample preparation, and more applicability under field condition, Vis-NIR instruments are more widespread than MIR, although the latter, being less affected by water content, particle size, and light penetration than the former, generates more accurate predictions and measurement reproducibility (Reeves et al., 2010).

Building upon the approaches successfully developed for field and laboratory spectroscopy in the reflective (Vis-NIR) and thermal (MIR-TIR) domain, imaging spectroscopy using airborne sensors has shown the potential to map and to quantify topsoil properties (e.g., Stevens et al., 2010; Eisele et al., 2012; Gerighausen et al., 2012). Upcoming spaceborne sensors will cover not only the Vis-NIR region (EnMAP from Germany, HSUI from Japan, PRISMA from Italy, and SHALOM from Italy and Israel) but also the thermal infrared (TIR) region (HyspIRI). These instruments, contributing to, e.g., the COPERNICUS/GMES programme, could therefore provide global spectroscopic data for mapping soil properties at low costs.

3.3 Cost/Benefit Analysis of Soil Spectroscopy

Besides the previously mentioned advantages, soil spectroscopy is also characterized by the fast delivery of results, and the need for only basic analytical infrastructure. For many soil assessment and monitoring applications, overall accuracy might be improved by obtaining soil data in more locations, depths, and times despite reduced accuracy for each measurement. O'Rourke and Holden (2011) calculated the costs per sample, analytical accuracy, and time involved in SOC analysis, in order to identify the best method among Walkley-Black, total organic C (TOC) analyzer, Vis-NIR diffuse reflectance spectroscopy, and laboratory hyperspectral imaging. They found that Vis-NIR spectroscopy and laboratory hyperspectral imaging (800–1720 nm), although less accurate than TOC analyzer, outperformed the latter, mainly as a result of the lower price (10 times cheaper). Schwartz et al. (2012) showed that the use of spectral analysis to monitor petroleum contamination in soils provided much better results at lower cost compared to certified laboratories using traditional analytical chemistry methods. This difference of cost is even more important for large-scale soil assessment, where the number of measurements is very high. The World Agroforestry Centre (ICRAF) reports that the price of SOC analysis by spectroscopy for the African soil information system (AfSIS) project, is one-third of that by a C/N analyzer. The Joint Research Centre of European Commission contracted a laboratory to analyze about 20,000 samples for the land use/cover area frame survey (LUCAS) soil monitoring network (Tóth et al., 2013). For SOC analyses, the cost per sample using spectroscopy was less than one-third compared to reference analyses (Soil Service of Belgium, pers. Comm.).

3.4 Spectral Libraries: State of the Art and Potential Use

Predictions are obtained by calibrating spectral data against reference soil analytical data. Spectroscopic calibrations are empirical and hence cannot produce accurate predictions for samples not represented in the spectral libraries. The usefulness of spectral libraries covering and producing prediction models for small areas has already been proven, and is not discussed in this paper. Hence, we focus on large spectral libraries, which are needed to provide general and robust models over large areas that are characterized by a large soil diversity. Below, we describe the characteristics of existing spectral libraries covering large areas.

The ICRAF-ISRIC world soil spectral library is composed of 4438 samples from 785 soil profiles distributed in 58 countries from Africa,

Asia, Europe, North America, and South America selected from the Soil Information System (ISIS) of the International Soil Reference and Information Centre (ISRIC) archives. Samples were scanned in the Vis-NIR spectral range (350–2500 nm), with a FieldSpec FR spectroradiometer (Analytical Spectral Devices, Boulder, CO). The soil reference measurements were acquired by ISRIC in different laboratories according to ISRIC Procedures for soil analysis (Van Reeuwjik, 2002).

Viscarra Rossel and Webster (2012) described a large library of 21,500 Vis-NIR spectra from around 4000 soil profiles covering the Australian continent. The soil samples were scanned using an ASD LabSpec Pro spectrometer with a spectral range of 350–2500 nm and spectral resolution of 3 nm at 700 nm and 10 nm at 1400 and 2100 nm. The collection of soil spectra was realized with a high-intensity contact probe with halogen bulb illumination. The samples were collected from different soil surveys conducted at different scales (continental, regional, and farm). The soil analyses were realized in multiple laboratories, following different analysis protocols.

A spectral library covering the United States has been collected under the Rapid Carbon Assessment project (USDA, 2013). The library is composed of 144,833 Vis-NIR spectral scans, derived from samples collected from the upper 1 m of 32,084 soil profiles at 6017 randomly selected locations. The instrument used to scan the samples was an ASD LabSpec Pro spectroradiometer with a spectral range of 350–2500 nm, 2 nm sampling resolution and spectral resolution of 3 nm at 700 nm and 10 nm at 1400 and 2100 nm. Soil spectra were acquired using a high-intensity contact probe. SOC was determined by combustion method.

The European spectral library LUCAS consists of about 20,000 topsoil (0–20 cm) samples, collected from all over Europe, measured for 13 soil properties in a single laboratory (Stevens et al., 2013). The Vis-NIR soil spectra were measured with a FOSS XDS Rapid Content Analyzer (FOSS NIRSystems Inc., Denmark), operating in the 400–2500 nm wavelength range, with 2 nm spectral resolution and 0.5 nm spectral data interval.

In addition to continental-scale libraries, a number of national and regional soil spectral libraries have been constructed, such as the ones for France (Gogé et al., 2012 for NIR and Grinand et al., 2012 for MIR), Czech Republic (Brodsky et al., 2011), Denmark (Knadel et al., 2012), Florida (Vasques et al., 2010), and Brazil (Bellinaso et al., 2010).

Soil spectral libraries might be a strong base for the forthcoming hyperspectral remote sensing of soils from space. The laboratory soil spectra may enable appropriate validation of the reflectance information extracted from

radiance data acquired from remote platforms. Moreover soil spectral libraries can play a major role in tracking temporal spectral changes over the sampling locations (Deng et al., 2013).

3.5 Soil Spectroscopy for Large-Scale Soil Property Prediction

Predicting soil properties for large and diverse areas is especially challenging, and results in higher prediction error than for local scale spectroscopic models (Stevens et al., 2013). For instance, Brown et al. (2006) obtained a root mean square error (RMSE) of 7.9–9.9 g C kg^{-1} for an SOC content calibration model obtained from samples distributed all over the world. In Europe, spectral models of SOC content achieved an RMSE of 3.6–8.9 g C kg^{-1} for mineral soils and 50.6 g C kg^{-1} for organic soils (Nocita et al., 2014; Stevens et al., 2013). Vasques et al. (2010) developed spectroscopic models of SOC in Florida achieving an RMSE of 6.5–7 g C kg^{-1}. These prediction errors are large compared to the standard error of laboratory (SEL) of established methods of soil carbon analysis, such as dry combustion (SEL = 1–2 g C kg^{-1}; Gerighausen et al., 2012). Large-scale libraries tend to span over a wider range and a higher variability of the soil property under study, which actually appear to be the dominating factor influencing prediction errors (Stenberg et al., 2010). The lack of accuracy shown by spectroscopic models built with large-scale spectral libraries is also due to the complexity of the relationship between soil properties and spectra for heterogeneous soil samples. It has been long recognized that the spectral signature of soils in the Vis-NIR region is not unique (Price, 1994). Many absorption features overlap so that absorptions related to one soil component can be masked, distorted, or shifted where other soil components vary. For instance, spectral variations related to changes in iron oxide content may cancel variations in absorptions due to organic matter (Adar et al., 2014). Not only chemical chromophores interact with each other. For instance, for the same amount of SOC content, an increase in sand content induces an increase in SOC absorption depths, which can be easily confounded with an increase in SOC (Stenberg, 2010; Stevens et al., 2013). To overcome this, it has been proposed to include additional variables such as particle size distribution in the modeling of soil spectra (Brown et al., 2005; Nocita et al., 2014).

The use of large spectral libraries has been proposed also for field and farm scale soil mapping. In order to overcome the large biases often experienced when using them at the local scale, the spiking technique, consisting

in the combination of large-scale libraries with local calibrations, was developed (Guerrero et al., 2010). Basically only a handful of local samples are included in a large-scale library to shift its weight towards the target site. In this approach, a few representative samples from the target site (spiking subset) are added to recalibrate the model, ensuring that the models contain samples similar to those to predict. This approach implies some analytical efforts, since the spiking subset must be analyzed with the reference method. Consequently, this subset should be as small as possible, in order to maintain most of the advantages of spectroscopy.

The poor calibration results at large scales are not only due to the geographical extent and the resulting complexity as such, but also to the sampling density of the current spectral libraries. Based on the LUCAS spectral library (∼20,000 points across Europe), Nocita et al. (2014) showed, for instance, that, using a local regression approach (i.e., for each spectrum to predict, a calibration equation is developed based on the samples with the most similar spectra or the closest samples in the geographical space), soils with high SOC content (80 g C kg^{-1}) were poorly predicted because of the lack of suitable nearest neighbors (i.e., samples with similar soil and spectral properties to the ones to predict). Generally, the analyses of such large databases need dedicated chemometrics tools, such as local regressions that are able, to some extent, to handle their complexity (Ramirez-Lopez et al., 2013). Development costs of large databases are high, so that such spectral libraries are often developed from archived soil samples and legacy soil databases with analytical measures (Viscarra Rossel and Webster, 2012). Although these libraries contain an enormous wealth of information on soils, they cannot be easily merged into a uniform database because they have been collected with different protocols, instruments, and analytical methods which can severely affect the prediction performance of spectroscopic models (Soriano-Disla et al., 2014). For instance, Brown et al. (2005) computed an RMSE of 6 g C kg^{-1} for 1175 samples analyzed for SOC both by dry combustion and the Walkley-Black method. Such error would be included in the total error budget of SOC spectroscopic models based on samples analyzed with the two different methods. Obviously this represents a waste of resources since most spectral libraries cannot be exploited together to create robust models over large areas and with diverse soil types.

3.6 Parameters Causing Spectral Variation in the Laboratory

Not only the soil components but also the laboratory protocols have an influence on the spectra. Depending on the instruments, samples are prepared

following a procedure specific to each laboratory, which may result in difference in spectral shapes for the same sample between different laboratories. Often, soil samples are dried, sieved, and grinded. Differences in water content of air-dried samples, due to fluctuations in relative humidity of the ambient air in the laboratory, affect the spectral shape and peaks, especially around 1.415 and 1.915 μm (Whiting et al., 2004; Nocita et al., 2012). Spectral reflectance is also affected by the grinding of soil. This can generate important differences of accuracy in the prediction models due to the variation of particle sizes (Soriano-Disla et al., 2014). Unfortunately, there is no protocol specifying at which caliber samples should be grinded. The same is true for sieving. This makes the construction of comparable spectral libraries difficult, and precludes the sharing of spectra among laboratories with different measurement conditions and protocols. Pimstein et al. (2011) showed that the use of a common protocol and an internal standard reduced significantly the differences between spectral measurements of the same samples by different operators in three laboratories.

The comparison of analytical results among laboratories is traditionally addressed by performing ring tests with a standard sample being sent and analyzed by all participating laboratories. For spectral analysis, it is recommended to send both a reference material, such as bleached inert sand, and a standard soil sample to the laboratories. The spectra of these materials can then be used to determine transfer function for the spectra determined in one laboratory to be used in a spectral library of another laboratory.

3.7 Metadata and Soil Spectroscopy

Metadata are structured information that make an information resource easier to access, use, and understand. Just as for any other kind of information derived in the field or the laboratory, the utility of soil spectroscopy data is only as good as how well it can be explained and understood. Capturing the metadata at the observation stage is a lot more cost-effective than trying to work out all of the necessary information later on, and can aid in the integration of the data within global frameworks, and its later extraction and use.

Many different definitions and frameworks for metadata have been developed, reflecting the wide variety of data and information types that can be generated. Existing examples of metadata standards of relevance to soil spectroscopy include ISO 19115 (ISO, 2003), the Dublin Core Metadata Initiative (DMCI, 2013), and the Directory Interchange Format (NASA, 2013).

The seven questions that the notion of metadata addresses are all relevant to soil spectroscopy, and are listed below:

- *What* was measured? This can include the spectroscopic information, additional environmental parameters, and soil analysis results obtained later in the lab.
- *Who* carried out the measurements? Some field observations and laboratory analyses are operator-dependent, and it can be useful to know who carried out the work in order to calibrate for this (and also to be able to ask them questions about the data if the need arises).
- *Where* were the measurements made? Geographical location of field observations can allow later site characterization information to be derived.
- *When* were the measurements made? This information is particularly important when long-term monitoring or changes over time are of interest.
- *How* were the measurements made? Type of equipment, field sampling protocols any other seemingly innocuous information about how the data were derived can be useful later.
- *Why* was the work carried out? Was it in relation to some other project, and were the observations intended to satisfy some specific requirement?
- *Whose* is the data? Intellectual property is a topic that tends to be ignored until it becomes an unavoidable issue—having information about ownership, rights of use, and referencing of the material available to the user early on can facilitate license negotiations and prevent problems occurring once work has been carried out.

Suggestions for implementation of metadata standards within soil spectroscopy are likely to cover a wide range of topics, from the adoption of preferred standards to the inclusion of specific types of information (and the formatting of the metadata framework). There is a great deal of flexibility available in how such a system could be adopted, and we are not attempting here to prescribe how this should be achieved. However, any successful metadata framework should aim at the very least to be compliant with the INSPIRE directive (http://inspire.ec.europa.eu/), and it is proposed that the information and guidance available in relation to this directive would be a suitable starting point for discussions.

4. THE WAY FORWARD

The growing demand for high-resolution soil data to cover large areas on the one hand, and the lack of availability of such data on the other hand,

is one of the biggest challenges in contemporary soil science (Grunwald et al., 2011), encouraging thereby the development of cost-effective methods of soil analysis, such as Vis-IR spectroscopy. While research on soil spectroscopy has rapidly grown and showed a great potential (Guerrero et al., 2010), it is now time for soil spectroscopy to enter an operational phase where, just as for other established soil analytical techniques, measurements are standardized, soil analyses are reliable across diverse environments and data are delivered in an automated mechanism. To achieve this goal, one prerequisite is the development of databases that can provide robust spectral models over large geographical extents. It is unlikely that the high sampling density, required to appropriately describe soil variation at these scales, could be reached by a single research group. However, the combination of existent and future local, regional, and continental spectral libraries is an achievable target, provided that they are built using a common protocol for the collection of laboratory soil spectra, or that they contain spectral reference measurements so that spectral transfer functions could be calculated. Similarly to spectral measurements, the use of reference analytical methods that spectroscopic predictions rely on should be standardized. Obviously, a joint effort of the soil spectroscopy community is required to allow better interoperability between soil spectral libraries and facilitate data exchange. Such initiative is not only a way to unlock current limitations of soil spectroscopy, but also a lever that would directly promote the use of spectroscopy in academic and commercial soil laboratories, favoring the development of monitoring networks able to assess quickly and efficiently the state of the soil resources at minimal costs. In the next sections, we present some ideas that, once implemented, could contribute to the expansion of soil spectroscopy as an established soil analytical method.

4.1 Establishment of a Common Protocol for Laboratory Spectroscopy

Since calibration and management represent a large part of costs and efforts in the use of IR techniques, standardization and centralization of reference methods are strongly needed. This represents the first step for the sharing of small- and large-scale spectral libraries, which could help in achieving calibration models that are valid for larger areas. So far, all the national and continental spectral libraries have been built using slightly different protocols for each library including sampling technique, sample preparation, instrument specifications, and spectral acquisition, which hinder interoperability of spectral libraries. The use of different reference methods and the problems

related with quality control of reference measurements within and across laboratories provide major challenges for the development of reliable calibrations. Iterative development of centralized spectral libraries is efficiently achieved by screening spectral libraries and then conducting reference measurements on outlying samples. An international standard for the collection of laboratory spectra and the inclusion of spectra of reference materials will dramatically drop the costs linked with the collection of new samples. Moreover, the development of a common standard, together with a common network for scientists and technicians from all over the world, will give rise to new applications, such as the transfer of calibration models from laboratory to spectra of the soil surface collected by remote sensing.

4.2 Scanning of Existent Soil Archives

Sampling campaigns are costly, and soil archives stored by universities, research centers, agriculture associations, and government agencies could provide an opportunity to enlarge spectral libraries. Even if many samples were acquired decades ago, they still contain spectral information that could be used to improve the representativity of spectroscopic calibration models. These samples could be scanned to a cost basically corresponding to the working hours of a technician. In many cases, however, considerations on the property of reference data and on the confidentiality of some metadata (especially location) have to be addressed. A common framework could be proposed for negotiating with data owners (e.g., access to spectra, and imprecise geographical coordinates). The impact of this mobilization, realized following a common protocol, might be part of the answer to the demand for robust calibration model across regions and soil types. Since 2006, ISO/TC 190 (soil quality)/SC 3/WG 10 (screening methods) has been developing standards to screen soil for chemical compounds including heavy metals, petroleum, the total carbon, and nitrogen as well as harmful anions such as chromium (VI) and cyanides, under the guidance established in 2011 as ISO 12404 on screening methods to be applied to soil monitoring.

4.3 Storing Spectra and Associated Soil Archives

The spectrum of a soil sample contains abundant information. Once scanned, a spectrum can be stored easily. It is probable that, in the future, soil scientists might be measuring some properties which at the present time are not measured, because neither the knowledge nor appropriate analytical techniques are available. The characteristics of the soils stored as spectra will remain unaltered, while soil properties will change after a long

storage. This is why soil spectral libraries should be accompanied with soil banks that conserve the soil samples to be scanned or analyzed in the future.

4.4 Spectroscopy to Acquire Standardized Soil Information and Enhance Monitoring

The implementation of soil spectroscopy could find valuable application in the soil information system and would represent a great progress in the field of soil analysis. For instance, soil classification can be realized by several methods (Soil Survey Staff, 1999; IUSS Working Group WRB, 2006), but the high costs for its implementation has hindered the collection of this information. Soil spectroscopy can be applied for the classification of soil types (Viscarra Rossel and Webster, 2012). Demattê and Terra (2014) demonstrated the potential of spectroscopy for the evaluation of the changes in soil type along topo-sequences, as a basic tool for soil mapping. Moreover, Demattê et al. (2004) showed that soil spectroscopy can be used to map soil types, as the basis for land-use planning.

At the same time, soil spectroscopy could be integrated and thus regulate the use of fertilizers based on routine soil testing. For example, one of the main problems of a reliable fertility status is the lack of knowledge of the CEC and clay content of our soils. Indeed, those properties are the key to compare samples to the regional reference system and deliver an appropriate agronomic diagnosis. The determination of CEC and clay content using standard procedures is not feasible for each soil sample as it is too costly and time-consuming. An alternative is to predict them with Vis-NIR spectroscopy which represents thereby a real opportunity to improve the fertility advice (Genot et al., 2011). In Wallonia, these analyses are routinely carried out since 2008 in the laboratories of the http://www.requasud.be/. Another example of soil spectroscopy application for routine analysis is the AfSIS. They have adopted Vis-IR spectroscopy as its main screening tool in characterizing 20,000 soil samples taken from a stratified random sampling frame across Sub-Saharan Africa. There is a growing network of Vis-IR laboratories in Africa, with more than 10 laboratories established, including two private sector soil testing companies.

Recently soil spectroscopy was reported as an accurate method to monitor temporal changes in SOC of Danish soils (Deng et al., 2013). Basically, this study used topsoil (0–25 cm) samples from 1986 to 2009. Spectra collected from both time series revealed that Vis-NIR spectroscopy could soundly detect the SOC temporal decrease observed with wet chemistry, but at much lower cost.

In conclusion, given the adherence to a common protocol, spectroscopic analyses could both increase the reliability and the comparability of the results and, at the same time, contribute to the construction of the spectral libraries. The latter requires quality assessment/quality control steps to be built in the database for the input of new spectra. Diffusing the use of Vis-NIR and MIR spectrometers at all levels, especially farmers' consortia and agriculture organizations, responsible for the soil analyses of thousands of samples collected over large areas, could bring important advantages. The long-term cost cuts from which these associations would benefit will no doubt justify the investment in instruments. Many regional laboratories already operate Vis-NIR spectrometers to infer soil properties. This implies that the establishment of local and regional partnerships among existent institutions will already generate enough competence for the soil monitoring based on soil spectra. The development of this kind of project requires the support of international organizations, such as the Food and Agriculture Organization (FAO) of United Nations and the European Commission's JRC, and their acknowledgment of soil spectroscopy as a valuable tool to integrate the established techniques of soil chemical analyses for the control of the state of soils (Clark and Roush, 1984).

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CHAPTER FIVE

Occurrence, Detection, and Molecular and Metabolic Characterization of Heat-Resistant Fungi in Soils and Plants and Their Risk to Human Health

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Contents

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Abstract

Heat-resistant fungi are often factors causing spoilage of heat-processed products. Contamination of agricultural raw materials is often a result of their contact with soil. Materials contaminated by spores of heat-resistant fungi can be a risk to consumers' health because of toxic metabolites (mycotoxins) produced by these microorganisms. Due to resistance of fungi to high temperatures they are able to survive industry pasteurization process. Therefore, the only way to prevent the growth of these microorganisms in the product is suitable selection of material by conducting tests for the presence of heat-resistant fungi. The use of traditional culture methods is long and, therefore, does not apply in the selection of raw materials for production. However, time is a critical factor in assessing the acceptance or rejection of a given batch of raw material, due to the necessity of processing it fresh, which is very important especially in the case of fruit.

1. HEAT-RESISTANT FUNGI: IMPORTANCE AND CURRENT OUTLOOK

Heat-resistant fungi contamination is a major problem for fruit-processing industry in many countries. Soft fruit sector, especially the production of strawberries, plays a very important role in the agricultural and horticultural production, not only in the European Union but also around the world. Statistics shows that Poland's strawberry production rate of 181.9×10^3 tons of fruit, and Spain (300.9×10^3 tons), and together with Turkey (186.1×10^3 tons) and Italy (161.0×10^3 tons) are the largest manufacturers of this sort of fruit in Europe. Another place falls to Germany, which provides an average of 130.9×10^3 tons a year of this fruit. Therefore, the production of high-quality raw material is essential for both domestic market and for export. Literature and our studies (not published) show the emerging problem of contamination of soft fruit, especially strawberries and their products by heat-resistant fungi, which can be a serious threat for human health.

Fungi are classified mainly by their morphologic characteristics, that is the way observations of ascospores and anamorph morphology are important for identifying heat-resistant fungi. It needs a long time to form ascospores, however, special knowledge and techniques are required to identify them by their morphologic characteristics. Therefore, rapid and simple methods of identification and discrimination of heat-resistant fungi, like *Neosartorya fischeri*, which is the most significant agent, are required in the food industry (Yaguchi et al., 2012). In the food industry, rapid detection and identification of microorganisms or contamination sources is required to take decisions concerning the loss of raw material and production lots. This also minimizes the costs of unnecessary storage. Conventional detection and identification of molds are based on cultivable isolates and assessment of morphological features. No universal medium exists and only a few media are widely accepted for fungal isolation (Randhawa et al., 2002; Hellebrand et al., 2006). These methods are time-consuming and labor-intensive. Alternative methods for the detection of fungal species are based on antibody like ELISA assay as well as on DNA methods like polymerase chain reaction (PCR) techniques (Yaguchi et al., 2012). Advantages, especially in PCR-based methods are in the specific detection of small amounts of target organisms by amplifying their DNA in a considerable short time frame. For the detection of fungal contaminants in food and raw materials and soil, molecular methods are not adequately implemented yet because there are only a few studies concerning this problem so far, but there is need to develop these detection techniques directly in the environmental samples like soil, raw materials, and products.

This paper includes a review of occurrence and detection of the most important heat-resistant fungal contaminants, especially *N. fischeri* and *Byssochlamys fulva* in the environment, using conventional and molecular methods and presents properties of heat-resistant fungi and their risk to human health.

2. SIGNIFICANCE OF HEAT-RESISTANT FUNGI FOR HUMAN HEALTH

Heat-resistant fungi and their occurrence in the environment have been known for long time. However, the increase in public awareness of the need to protect the environment and increase of the demand for healthy food lead to sketching the lines of research related to the development of technology, environmentally friendly crop and high-quality crops. Various

environmental factors, soil and climate conditions (such as drought stress) and anthropogenic factors can lead to disruption of the biological balance and form a niche that can be occupied by pathogenic microorganisms, including heat-resistant fungi. Under favorable conditions, pathogens intensively colonize plants, causing microbiological contamination, which can often lead to the accumulation of toxic metabolites—mycotoxins, that poses a threat to the health of consumers. Some species are able to produce mycotoxins among other such as patulin, byssochlamic acid, byssotoxin A, assymetrin, variotin, verruculogen, and fischerin that have toxic effect on the consuming organisms (Tournas, 1994). Moreover, heat-resistant molds are cause of pulmonary infection, allergies, osteomyelitis, endocarditis, keratitis, intoxication with volatile compounds, and carcinogenic and toxic influence (De Hoog et al., 2000). Innovative nature of recent trends is related to research on the effectiveness of detection methods of heat-resistant fungi in soil and agricultural raw materials. There is only a few research, data, and review on the occurrence of heat-resistant fungi in soils and on the degree of crops contamination by these pathogens. This review is in line with the latest global trends related to the acquisition of high-quality food. Therefore, a key issue is the description of appropriate selection of materials with high quality. Hence the need to assess the degree of risk of crops contamination by heat-resistant fungi and to develop and verify the effectiveness of methods for the detection of these pathogens. Review on the characteristics of the metabolic profile is also an innovative part of the proposed paper, and may be useful in the evaluation of conditions for development of these microorganisms.

3. THE OCCURRENCE OF HEAT-RESISTANT FUNGI IN SOILS AND AGRICULTURAL RAW MATERIALS

In general, fungi are widely distributed in the environment. The following fungi have low heat resistance: *Aspergillus*, *Fusarium*, *Penicillium*, *Mucor*, *Rhizopus* and can be inactivated in pasteurization process at temperature 70 °C for 10 min (Yaguchi et al., 2012). Heat-resistant molds have been recognized in several countries as pathogens that are able to spoil thermally processed fruit and fruit products (Beuchat, 1998; Dijksterhuis, 2007; Hull, 1938; Kikoku et al., 2008; Olliver and Rendle, 1934; Put and Kruiswiji, 1964; Samson et al., 2004; Tournas, 1994). These molds were isolated from frozen blueberries (Kikoku et al., 2008), mango and grape juices, and other raw materials (Rajashekhara et al., 2000). Fungi

belonging to the following genus *Byssochlamys, Neosartorya*, and *Talaromyces* were most frequently encountered (Dijksterhuis, 2007). *Byssochlamys*, *Neosartorya*, and *Talaromyces* species are recognized as spoilage molds in canned fruit, since 1934, 1963, and the early 1930s, respectively (Kavanagh et al., 1963; Olliver and Rendle, 1934). It is known that heat-resistant fungi are able to survive temperatures higher than 75 °C for 30 min (Pena et al., 2004). Most important molds are *Neosartorya*, *Thermoascus*, *Byssochlamys*, *Eupenicillium*, *Talaromyces*, *Monascus purpureus*, *Humicola fuscoatra*. Investigating this specific type of molds started in the 1930s (Hull, 1938) and is being studied till today. These microorganisms are important in the spoilage of heat-processed fruit products. The principal heat-resistant fungi belong to the genera *Neosartorya*, *Byssochlamys*, and *Talaromyces* (Ferreira et al., 2009; Sant'Ana et al., 2009), as presented in Figure 1. The main sources of these pathogens are fields and orchards. Houbraken et al. (2008) and Tournas (1994) reported that heat-resistant fungi are common in soil, indoor environments, plants, animals, and foodstuffs. These pathogens were also detected in paperboard packing materials (Delgado et al., 2012a,b). The soil is the principal contamination source of *Byssochlamys* and *Neosartorya* species. The heat-resistant fungi were found in soils over the world, such as India, Australia, Japan, Israel, Turkey, South Africa, Jamaica (Domsch et al., 1980), Egypt (Ali et al., 2009), Nigeria (Amaeze et al., 2010), Brazil (Horie et al., 2003), Japan (Someya et al., 1999), Korea (Hong et al., 2006), Slovakia and Czech Republic (Simonovicova et al., 2014), and

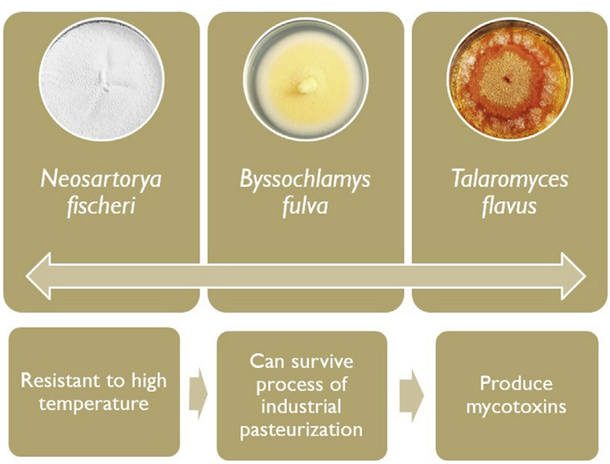

Figure 1 The most dangerous species of heat-resistant fungi and their importance.

Poland (Bilińska et al., 2013). The ascospores can remain dormant, survive the commercial pasteurization conditions normally applied to fruit products, and spoil such products by germinating and growing even under reduced oxygen conditions (Kotzekidou, 1997). The growth of these fungi can produce CO_2, which inflates fruit packages and causes visual deterioration of the products due to pectinolytic enzymes activity (Ugwuanyi and Obeta, 1999). Fruits having more contact with soil or dust are more susceptible to contamination by heat-resistant molds. Although low counts (10^1–10^2 CFU per 10 g) of these microorganisms have been reported in the soil (Jesenska et al., 1992), they are not higher than 10^1 CFU per 100 g in the fruits before processing (Sant'Ana et al., 2010). Ascospores of *B. fulva* and *N. fischeri* are extremely heat resistant and frequently associated with the deterioration of thermally treated fruit products (Hocking and Pitt, 1984). In order to ensure the microbiological stability of products, the knowledge of inactivation kinetics of heat-resistant mold spores as affected by heat is essential for the design of an effective pasteurization process conditions (Delgado et al., 2012a,b). Heat-resistant ascospores of *Byssochlamys* sp. are responsible for spoilage of strawberry, pineapple, passion fruit, mango, grape, and citrus fruit (Hosoya et al., 2012; Tournas, 1994) and also was found in silages (Tangni et al., 2013). Because *Byssochlamys* spp. are prolific under low oxygen conditions, they can reduce the quality of processed fruit food products by making them the most harmful organisms in acidic food products. The most important problem concerns the *B. fulva* and *N. fischeri*, which can grow on strawberry. Studies of many researchers (Eziashi et al., 2010; Frąc et al., 2013; Hosoya et al., 2012; Yaguchi et al., 2012) indicated that these species were isolated from fruits and also from fruit products, juices, nectars, and wines. *Neosartorya fischeri* is a ubiquitous fungus which commonly grows in such environments as soil and organic debris including fruit and vegetables, where it produces high numbers of spores (Nielsen et al., 1988). Ascospores of *N. fischeri* are thermoresistant and can cause food spoilages of heat-processed fruit products in food industry (Girardin et al., 1995; Salomao et al., 2007). These fungi were detected in the plantation of plants such as palm, sugar cane, rice, cotton, potatoes, barley, and banana (Malloch and Cain, 1972).

Hamigera and *Thermoascus* belonging to the order *Eurotiales* form ascospores that are highly heat resistant (Hosoya et al., 2014; Nakayama et al., 2010). The major species of *Thermoascus* genus includes *Thermoascus crustaceus*, *Thermoascus thermophiles*, *Thermoascus aurantiacus*, and *Thermoascus aegyptiacus* (Houbraken and Samson, 2011). These fungi were isolated from

different agricultural products including maize, olive and olive cake (Roussos et al., 2006), and food-related environments (Yaguchi et al., 1995). Species belong to this genus were detected in tea and fruit juices (Hosoya et al., 2014). It is a very big problem, because control of heat-resistant fungi by heat-pasteurization is difficult, and it is thus important to prevent contamination through monitoring of raw materials, soils under fruit cultivation, and manufacturing environment (Hosoya et al., 2012). The occurrence of heat-resistant fungi is also strongly depended on environmental factors such as temperature, water activity, pH, redox potential, oxygen concentration, and preservatives. The importance of microbiological quality of agricultural raw materials was presented at Figure 2.

4. THE EFFECTIVENESS OF DETECTION METHODS OF HEAT-RESISTANT FUNGI

The most common identification method relies on morphologic examination, but the high degree of similarity among these fungi and species from *Aspergillus* is very problematic for rapid and correct identification. There is a need to test modern identification methods of heat-resistant fungi

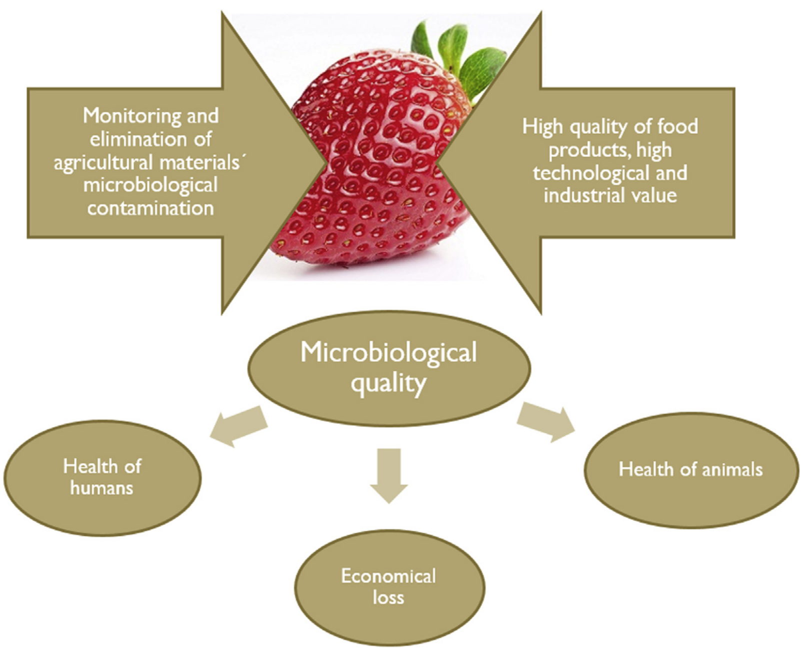

Figure 2 The importance of microbiological quality of agricultural raw materials.

based on molecular biology techniques. The most common gene used in molecular detection of heat-resistant fungi is β-tubulin gene (Hosoya et al., 2012). It is needed to evaluate risk and possibility of heat-resistant fungi detection in raw materials and soil using specific primers for this region in PCR. β-tubulin gene is widely utilized for phylogenetic classification of fungi at the species level (Balajee et al., 2009), but the most important part of analysis is appropriate optimization of DNA extraction procedure. This chapter includes complex research on the methodology of heat-resistant fungi isolation from soil and raw materials, taking under particular consideration the properties of the isolated microorganisms. Fungal identification was described based on conventional and molecular biology techniques, including DNA extraction and amplification with specific primers and PCR. In this chapter, the following problems are described: evaluation of effectiveness of molecular biology methods for detection of heat-resistant fungi. Figure 3 presents the detection methods of heat-resistant fungi.

4.1 Conventional Methods for Detection of the Heat-Resistant Fungi

Because of their low range in fruits, ascospores are not likely to exceed 1 to 10 per 100 g or mL of processed products, large samples should be analyzed for their effective detection. Centrifugation may be used to concentrate

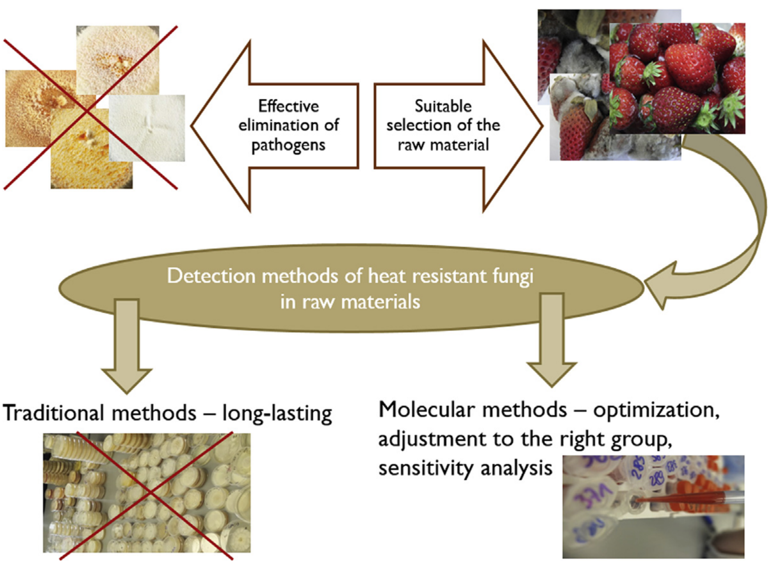

Figure 3 The detection methods of heat-resistant fungi.

ascospores in liquid fruit products. A second but very important point is that ascospores of heat-resistant fungi require heat activation before growth will occur (Beuchat and Pitt, 1992; Houbraken and Samson, 2006; Murdock and Hatcher, 1978; Samson et al., 2004). Different strains within the same species may require different treatment periods of time and temperatures to achieve maximal activation. Enumeration of heat-resistant ascospores relies on a selective heat treatment that inactivates vegetative cells of fungi and bacteria as well as less heat-resistant spores. Heat-resistant fungi are not fastidious in their nutrient requirements, and therefore the media such as potato dextrose agar (PDA), malt extract agar (MEA), or Czapek yeast autolysate agar can be used for their detection. When products are heavily contaminated with bacterial spores, media acidification or the addition of antibiotics to the plating medium may be required to inhibit the bacteria growth (Pitt and Hocking, 1997; Samson et al., 2009).

4.1.1 Plating Method

Fruit and fruit products should be homogenized using Stomacher bags. In this case, it is necessary to add 100 g of fruit or fruit products and 100 mL of sterile water and blend about 5 min. After homogenization, samples should be heated at 75 °C for at least 30 min. Two portions of homogenate (50 mL) have to be transferred to sterile test tubes and placed in a closed water bath at 75 °C for 30 min. After heating, homogenate should be thoroughly mixed and combined with PDA or MEA in petri dishes. Each 50 mL of sample should be equally distributed in four dishes and thoroughly mixed with 10 mL strong agar. Petri dishes should be incubated at 30 °C for up to 30 days. Most viable ascospores will germinate and form visible colonies within 7–10 days; however, some of them may require additional time to form colonies (Beuchat and Pitt, 1992). It is important to check the sugar concentration in the fruit products because if they have 35 °Brix or more, samples should be diluted (1: 2) with sterile water and thoroughly mixed before heat treatment.

The main problem associated with this technique is connected with the possibility of contamination plates with common fungi spores from the air. There is the risk that such contamination can give false-positive results (Pitt and Hocking, 1997). To minimize this problem, the plates should be prepared in the clean air or using laminar flow cabinet.

4.1.2 Direct Incubation Method

The method, which protects fruit samples against the air contamination is direct incubation method. This method is appropriate for fruit pulps and

also homogenates. As in the plating methods, it is necessary to homogenize the fruit product samples. In this case, 50 mL of homogenized sample is heated in 100-mL bottles at 80 °C for 30 min. Then, it is necessary to incubate samples at 30 °C for up to 30 days. In this procedure, there is no risk of airborne fungi spores contamination and this procedure minimizes loss of moisture. Colonies develop on the surface of the homogenate. The disadvantage of this method is connected with colony identification because each colony has to be picked and grown on suitable media. For cultivation and identification of fungi, the petri dish method is also recommended (Houbraken and Samson, 2006; Pitt and Hocking, 1997).

4.2 Molecular Biology Methods for Detection of Heat-Resistant Fungi

The most popular technique of filamentous fungal isolates identification and detection is microscopic demonstration and characteristics of its morphologic structures after growth on different selective media. Beside microscopic based methods, filamentous fungi have been traditionally identified based on phenotype of the organisms (Chen et al., 2011). However, such identification can be subjective and additionally inexperience in microscopy may lead to misidentification. Moreover, phenotype-based identification was inaccurate in comparisons to molecular identification of fungal strains (Chen et al., 2011; Frąc, 2012; Guarro et al., 1999). To solve these problems, many researchers carry out study concerning molecular-based methods in identification and detection of fungi (Chen et al., 2002; Geisen et al., 2004; Nakayama et al., 2013; Yang et al., 2004), especially heat-resistant fungi (Hosoya et al., 2014; Nakayama et al., 2010; Yaguchi et al., 2007). Figure 4 presents the most popular molecular biology methods used in heat-resistant fungi detection and identification.

Because of the fungal cell wall composition including chitin, glucans, lipids, or other polymers, which are resistant to enzymatic and chemical reagents, the first crucial step of molecular detection and identification of spoilage fungi is the DNA extraction (Chen et al., 2011). There are a variety of different methods for DNA isolation. These techniques have the great advantages that the spectrum of fungal species and their numbers present in food samples can be determined. For fungal DNA isolation, it is possible to use different methods such as biochemical, mechanical, physical, or their combinations (Karakousisa et al., 2006). The first type of methods—biochemical DNA extraction includes lysis buffer containing dodecyl sulfate (SDS) and proteinase K (Mahuku, 2004), SDS and

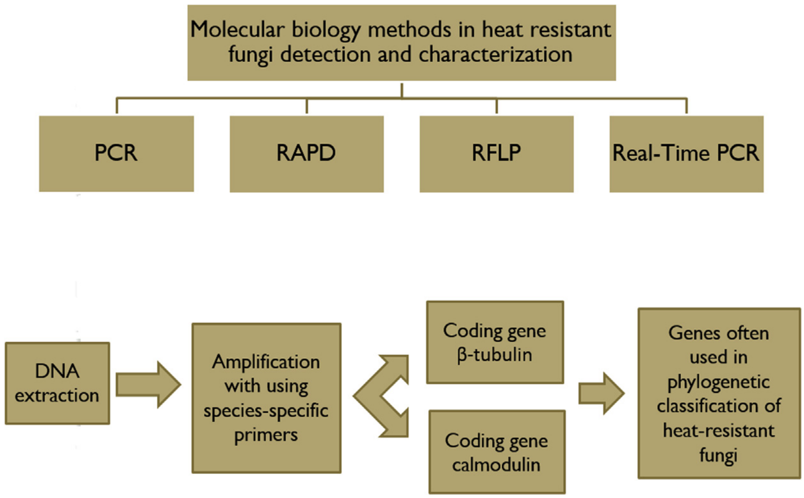

Figure 4 The most popular methods used in heat-resistant fungi detection and identification. PCR, polymerase chain reaction; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism.

spermidine (Borges et al., 1990), benzyl chloride (Zhu et al., 1993), alkaline chemicals (Bir et al., 1995), hexadecyltrimethylammonium bromide (Doohan et al., 1998), and also DNA isolation kit (Frąc, 2012; Hosoya et al., 2014). Mechanical methods are connected with different types of lysing matrix such as different size of glass beads, ceramic, and silica spheres combined with shaking or mechanical homogenization using special equipment (Dean et al., 2004; Frąc, 2012; Melo et al., 2006). Physical methods include the grinding with liquid nitrogen (Wu et al., 2001), microwave treatment (Tendulkar et al., 2003), and magnetic bead-based technology (Faggi et al., 2005) and are successfully used for fungal DNA isolation, but there is no study which compares the use of all these DNA extraction methods for heat-resistant fungi. Another important aspect is the availability of protocols to isolate DNA from food samples in a PCR-required purity. It is known that components of food can interfere with the PCR reaction (Rossen et al., 1992). Now we are working to develop and recommend the most suitable method for DNA extraction from heat-resistant fungal strains and food contaminated by such fungi.

4.2.1 Polymerase Chain Reaction

PCR methods in heat-resistant fungi diagnostic can be very valuable tools in fungi identification, especially for rapid detection of heat-resistant fungi in

the acidic food and beverage industry. PCR methods with specific primers are very precise and useful for the rapid detection of fungi and that is why molecular techniques have now become standard approaches in fungal identification (Chen et al., 2011). Internal transcribed spacer (ITS) regions of rDNA are efficient for species- or genus-level identification of most fungi and primers targeting the calmodulin gene in *Aspergillus* and *Neosartorya* identification. Calmodulin gene was also reported as a method of identifying ochratoxin-producing fungi (Susca et al., 2007). Increasing number of the heat-processed food and beverages spoilage by heat-resistant fungi had influence on the development molecular-based methods for such fungi detection. The study on microtubules composition of *Byssochlamys* fungal strains, one from the most heat-resistant fungi, indicated that globular proteins such as α- and β-tubulin are main components of microtubules. Because of low level of intraspecific DNA variation in the β-tubulin gene (total length of about 2.000 bp), this region is useful for species-level identification of different fungal genus, including *Byssochlamys* (Nakayama et al., 2010). The variation of PCR techniques is duplex or multiplex PCR techniques. The same melting temperature of primers for different species or genus could be allowed to make PCR reactions under the same conditions as duplex or multiplex PCR. A crucial point for functional diagnostic PCR is the availability of unique target sequences, because if such specific DNA sequence can be detected by PCR, it can be concluded that the fungus contaminates the sample. Genomics is much extended and whole genomes become accessible from various fungal species. It gives a chance to propose primer sequences, which can be checked for possible cross-hybridization by means of genomic database (Geisen, 2007). Conventional PCR can be used only to evaluate the presence of fungi, without information about cells or spores number in the sample and there is no information about micotoxins production. According to Nakayama et al. (2010), Hosoya et al. (2012, 2014), and Yaguchi et al. (2012), PCR methods of *Byssochlamys*, *Thermoascus*, and *Neosartorya* species, respectively are rapid and useful methods in harmful organisms identification in food products. Table 1 presented the primers for the most common fungal species belonging to the genus of *Neosartorya* and *Byssochlamys*. PCR methods may be extended to other heat-resistant fungi as rapid detection technique.

4.2.2 Real-Time Polymerase Chain Reaction

Real-time polymerase chain reaction (R-T PCR) is a useful technique to direct online monitoring and helps in the determination of the

Table 1 Selection of described molecular detection systems of heat-resistant fungi as food contaminants

Species	Targeted sequence	PCR product (bp)	Primer name	Primer sequences 5′⟶3′	Assay	References
Neosartorya						
N. fischeri	β-tubulin	361	Nfi3F	AGTCGTTGCATAGGAGGGATCTA	PCR	Yaguchi et al. (2012)
			Nfi3R	TCCCTCCCGAGGTCATACCAAAT		
N. spinosa	β-tubulin		Nspc1F	GGTTAGTAACTTTTTCTCGC	PCR	
			Nspc1R	TCGCATACCAGGGAAATCAA		
N. pseudofischeri	β-tubulin		Npf2F	GTGCGACCGTGTGCAAATGCT	PCR	
			Npf2R	GAATTCTGAGTACGGGTTAGCGGT		
N. hiratsukae	β-tubulin		Nhi1F	TGGAATATTAGGACCAGCCAGCAT	PCR	
			Nhi1R	TCGTGAGATGTTGCCGAAGTAGTTAA		
N. glabra	β-tubulin		Ngl_1F	TCGTCGTGGGTATAGCTAACAG	PCR	
			Ngl1R	TGAGATGTTGCCAGTAGTTTAAT		
Byssochlamys						
B. fulva	β-tubulin	250	B.fulva1F	AACAATTCTACAGGCAGGGC	PCR	Hosoya et al. (2012)
			B.fulva1R	TAGTGGTCGGGTCAGCGGA		
B. nivea	β-tubulin	300	B.nivea1F	ACAAGAGACAGGAAGAGCCT	PCR	
			B.nivea1R	TTCTTGCCGGCAGCCTAGGA		
B. lagunculariae	β-tubulin	400	B.lag1F	TCGAGACGTGAGATTGGGAA	PCR	
			B.lag1R	TGTTACCAGCACCGGACTGT		
B. zollerniae	β-tubulin	300	B.zol3F	TGTTACCAGCACCGGACTGT	PCR	
			B.zol3R	TGTTACCAGCACCGGACTGT		
B. fulva, B. nivea	β-tubulin	150	B1F	TTGGGACCAAACAAGAGACA	PCR	Nakayama et al. (2010)
			B1R	TGTGCACTTACACACCAGCA		
B. spectabilis	β-tubulin	200	Pae4F	GAGCACGGCCTTGACGGCT	PCR	
			Pae4R-1	GCATATGGAGCGTCCTTATC		

PCR, polymerase chain reaction.

generated PCR product. During reaction, it is possible to observe the fluorescence increase of the reaction mixture. The fluorescence increase is registered by using Sybr Green, which intercalates into the rising PCR product or by applying the TAMRA or TaqMan probes. As filamentous fungi consist of different cell types like vegetative spores, ascospores, and hypha, the ratio between genome and cells number is not like 1:1. It is known that hyphal cells are multinucleate, but spores can be uni- or multinucleate depending on the fungal species. This observation complicates the interpretation of R-T PCR data and suggests checking correlation between such data and fungal growth (Geisen, 2007). In general, the copy numbers in R-T PCR are higher than the numbers of cells determined by the plate count method (Mayer et al., 2003). On the one hand, this can be explained by the multinucleate status of the fungal cells and on the other using R-T PCR it is possible to detect the dead cells or released DNA. R-T PCR was used as accurate, reliable, and high throughput quantification of target fungal DNA in environmental samples like soil, water, air, food, and also in the study of fungal diagnosis and epidemiology. Moreover, this technique is increasingly applied as a useful tool to the study of changes in expression genes in response to phytopathogenic and antagonistic fungi (Schena et al., 2004). R-T PCR, in significant way decreases the risk of false-positive results due to the lack of the gel electrophoresis step. This method is very sensitive, mostly higher than culture. The modification of R-T PCR technique is a reverse transcriptase method that can be used for gene expression monitoring (Geisen, 2007). This system seems to be very useful to analyze the activity of mycotoxins biosynthetic genes in relation to food safety, especially for toxins produced by heat-resistant filamentous fungi. R-T PCR was used to amplify a fragment of fungal mitochondrial cytochrome b genes for *Aspergillus fumigatus* (Spiess et al., 2003) and heat-resistant clinical isolate of *N. fischeri* (Weldhagen et al., 2008). R-T PCR amplification and Sybr Green detection produced positive results for clinical isolate as wee as for control strains. The results obtained for *N. fischeri* indicated close relationship between *A. fumigatus* and *N. fischeri*. Such grouping is supported by previous study, which suggested that *N. fischeri* and *A. fumigatus* may be considered as sister taxa with good branch support (Rydholm et al., 2006). Till now there is not much study on R-T PCR in relation to heat-resistant fungi detection. We started such study for *N. fischeri*, *B. fulva*, and *Talaromyces flavus* in the scientific projects

concerning molecular and metabolic characterization of heat-resistant fungi.

4.2.3 Restriction Fragment Length Polymorphism

Restriction fragment length polymorphism (RFLP) analysis is used to identify the changes in the genetic sequence that occurs after digestion using restriction enzyme. RFLPs can be used to trace inheritance patterns, identify specific mutations, and for other molecular genetic techniques (Saikia and Kadoo, 2010). Restriction enzymes are proteins isolated from bacteria that recognize specific short sequences of DNA and cut the DNA at those sites. Restriction fragments length polymorphism was used to genetic characterization of *Fusarium* isolates (Mes et al., 1994). The study of Attitalla et al. (2004) indicated that RFLP technique was sensitive enough as an efficient diagnostic tool for routine differentiation of *Fusarium* strains. PCR-RFLP allows rapid detection and identification of pathogens and is widely used for detection of variety pathogens due to its rapidness, sensitivity, and specificity (Saikia and Kadoo, 2010). RFLP was also successfully used in the identification of the genomic relationship between *A. fumigatus* and *N. fischeri* and between different varieties of *N. fischeri*, which cause spoilage of heat-processed fruit products and juices. EcoRI RFLP patterns was useful tool for differentiation of most strains of *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa*. On the other hand, all *N. fischeri* var. *fischeri* had the same RFLP pattern which was similar to that one of *A. fumigatus* (Girardin et al., 1995). RFLP method can be also used to localize the origins of fungal contaminations. It would be possible by comparison of *N. fischeri* strains isolated from soils where fruits were harvested with strains from fruits used in the industrial process and strains from contaminated processed products. RFLP was successfully used in the evaluation of geographical location and thermoresistance, which vary significantly among different strains (Nielsen and Samson, 1992).

The modification of RFLP technique is terminal restriction fragments length polymorphism (TRFLP), which was originally applied as a method for genotyping mixed DNA samples (Liu et al., 1997) and is currently developed in fungal community ecology (Avis et al., 2006; Dickie and FitzJohn, 2007; Yu et al., 2009). TRFLP profiling is based on amplification of the DNA mixed genetic material with fluorescently labeled primers. Next step includes digestion using restriction enzymes and sizing the labeled terminal fragments in genetic analyzer. The differences in the positions at which restriction enzymes cut DNA is thought to provide enough

variability for such DNA mixtures to be characterized and the organisms contributing to be identified (Alvarado and Manjon, 2009). This method has also some disadvantages and difficulties connected with ability to generate sufficiently different fragment size, the success of digestion, the dependency on the detection threshold of the genetic analyzer, and appropriate DNA sizing (Blackwood et al., 2007).

Due to the usefulness of RFLP-based techniques, it is necessary to develop such typing system to localization of the origins and the monitoring of heat-resistant fungal contaminants in food-industrial environments.

4.2.4 Random Amplified Polymorphic DNA

Random amplified polymorphic DNA (RAPD) analyses is based on PCR amplification of the pathogen genome with short arbitrary sequences that are used as primers. These primers are helpful in finding distinct complementary sequences and are able to produce specific pattern of bands. RAPDs rely on fingerprint technique and can be useful in distinguishing fungal species' varieties or strains (Capote et al., 2012). RAPD results can be useful for analysis of the genetic diversity among strains as well as among fungal populations. This technique was used to analyze the genetic diversity among different *Fusarium* species and races (Arici and Koc, 2010; Lievens et al., 2007). RAPDs were also developed as a method for identification and molecular characterization of fungal strains belonging to *A. fumigatus*, which are close related to *Neosartorya* heat-resistant genus (Brandt et al., 1998; Hong et al., 2005). Because of accurate and timely identification of heat-resistant fungi, this method is extremely important to minimize the economical cost and to protect human health. That is the way the RAPD utility is potentially useful as rapid alternatives to traditional microscopic and phenotypic identification method for filamentous fungi. *Neosartorya* species are taxonomically most closely related to *A. fumigatus*. RAPD technique confirmed previous molecular taxonomic evidence of variation among *A. fumigatus* and *Neosartorya* species (Brandt et al., 1998). RAPD-PCR method seems to be useful strategies for detection and identification of heat-resistant fungi. Moreover, results obtained from RAPD patterns are easy to interpret as they are based on amplification or nonamplification of specific DNA sequences (Capote et al., 2012). Due to rapidness and inexpensiveness of this technique and also lack of any prior knowledge of the DNA sequence of the target organisms, this method appears as a promising tool in laboratory identification and taxonomic classification of filamentous fungal strains (Nayaka et al., 2011), especially heat-resistant fungi.

5. CHARACTERIZATION OF METABOLIC PROFILE AND PHYLOGENETIC ANALYSES OF SELECTED HEAT-RESISTANT FUNGI

The characterization of metabolic profile and phylogenetic analyses of selected heat-resistant species (*N. fischeri* and *B. fulva*) will be presented in this chapter. Metabolic profile characterization of heat-resistant fungi was described based on our study concerning the ability to metabolize different compounds using phenotype microarray (PM) technique, Biolog carbon sources utilization database of filamentous fungi and literature data (Varga et al., 1997).

Malloch and Cain (1972) established the *Neosartorya* genus (family *Trichocomaceae*) to accommodate teleomorphs of species belonging to *Aspergillus fischeri* series of the *A. fumigatus* species group (Raper and Fennell, 1965). Section Fumigati includes 26 *Neosartorya* species and nine anamorph species (Hong et al., 2006; Horie et al., 2003). To clarify the evolutionary relationships among these species the following methods such as carbon source utilization spectra and isoenzyme patterns (Varga et al., 1997), phylogenetic relationships by sequencing parts of β-tubulin (Varga et al., 2000), and hydrophobin (Geiser et al., 1998) genes were used. Phylogenetic analysis and carbon source utilization data indicated close relationship between *A. fumigatus* and *N. fischeri* species (Varga et al., 1997, 2000; Yaguchi et al., 2007). Using conventional methods, *N. fischeri* cannot be efficiently discriminated from *A. fumigatus*, the human pathogenic fungus and has been considered as the putative sexual stage of this fungus. Genomic tools have been very useful in the investigation of the relationships between *N. fischeri* and *A. fumigatus* (Girardin et al., 1995). Peterson (1992) showed that *A. fumigatus* and *N. fischeri* species are closely related. Certain strains of *N. fischeri* are able to produce terrain, fumitremorgins A and B, and verruculogen, which is toxic and act on the central nervous system (Nielsen et al., 1988).

Byssochlamys has *Paecilomyces* anamorph, which was erected to accommodate single species *P. variotii*. The following species: *B. fulva*, *Byssochlamys nivea*, *Byssochlamys zollemiae*, and *Bverrucosa verrucosa* were added to this genus (Samson, 1974; Samson and Tansey, 1975). Recently, phylogenetic analysis of the ITS region, parts of the β-tubulin and calmodulin genes revealed that *Byssochlamys* genus includes nine species: *Byssochlamys fulva*, *Byssochlamys laguncularíae*, *Byssochlamys nivea*, *Byssochlamys spectabilis*, and *Byssochlamys zollerniae* (from a teleomorph) and *Paecilomyces brunneolus*, *Paecilomyces*

divaricatus, *Paecilomyces formosus*, and *Paecilomyces saturates* (anamorph) (Hosoya et al., 2012; Nakayama et al., 2010; Samson et al., 2009).

Recent study of Hosoya et al. (2014) indicated that *Thermoascus* species are closely related to patulin producers belonging to *Byssochlamys* genus. The detection of homology of the gene that encodes isoepoxydon dehydrogenase (idh) and the enzyme important in patulin production is a useful tool to evaluate the risk of this mycotoxin (Hosoya et al., 2014; Puel et al., 2007). Heat-resistant fungi can utilize a lot of different substrates, like carbohydrates, amino acids, carboxylic acids, and polymers. Some of them are very important for their growth and protection. Besides the differences in metabolic profile between genus and species, we observed huge differences in growth on various osmolytes between two *N. fischeri* strains: environmentally isolated from fruit-based product (Frąc et al., 2013) and coming from international collection DSMZ (DSM3700). The environmental strain grew on almost all osmolytes, whereas the growth of DSMZ strain was inhibited by most of them. Among 95 of different osmolytes, only 6–7% urea and 50, 100, and 200 mM sodium benzoate (pH 5.2) inhibited the growth of environmental strain (Figure 5). Figure 6 presents different carbon sources utilized by *N. fischeri*, *B. fulva*, and *T. flavus* using FF Biolog Plate (Table 2).

Heat-resistant fungi are characterized by the production of ascospores or similar structures with heat resistance. That is the way they are able to survive the thermal processes given to some fruit products. Some authors (Albrecht et al., 2010; Salomao et al., 2007) indicated that mannitol (the acyclic sugar alcohol), one of the most abundant solutes in nature, is important in heat-stress protection of fungal ascospores. Wyatt et al. (2014) suggested that mannitol could serve as an energy source and is quickly degraded after breaking dormancy of ascospores. Mannitol accumulated in the solution could play a role in prolonged ascospores maturation outside the cleistothecia (Dijksterhuis and Teunissen, 2004). Germination of ascospores may result in visible growth of mycelia on fruit and fruit products. Such fungi can also produce the pectinolytic enzymes causing complete breakdown of texture in fruits (Hosoya et al., 2012).

6. MYCOTOXINS PRODUCTION BY SELECTED HEAT-RESISTANT FUNGI

This chapter concerns with the assessment of the ability to toxin production by selected heat-resistant fungal species. The importance of

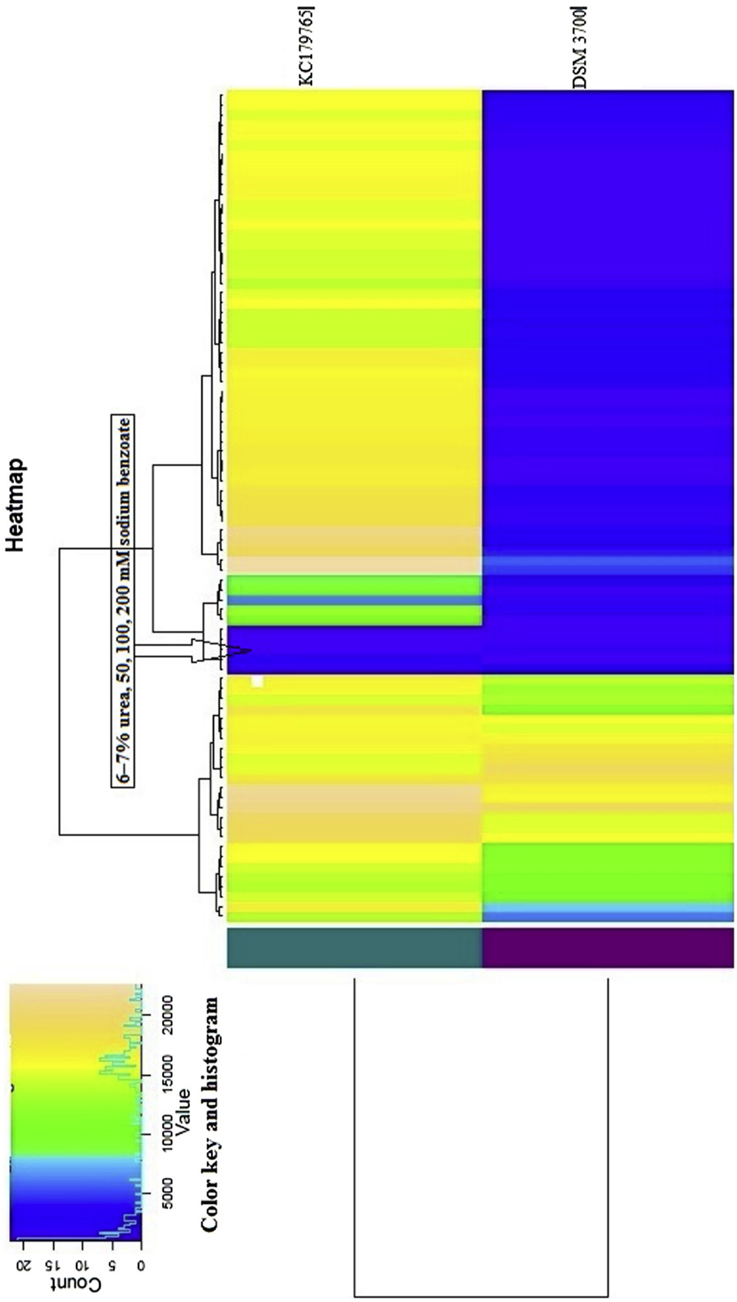

Figure 5 Phenotype Microarray based on PM9 Plates—osmolytes.

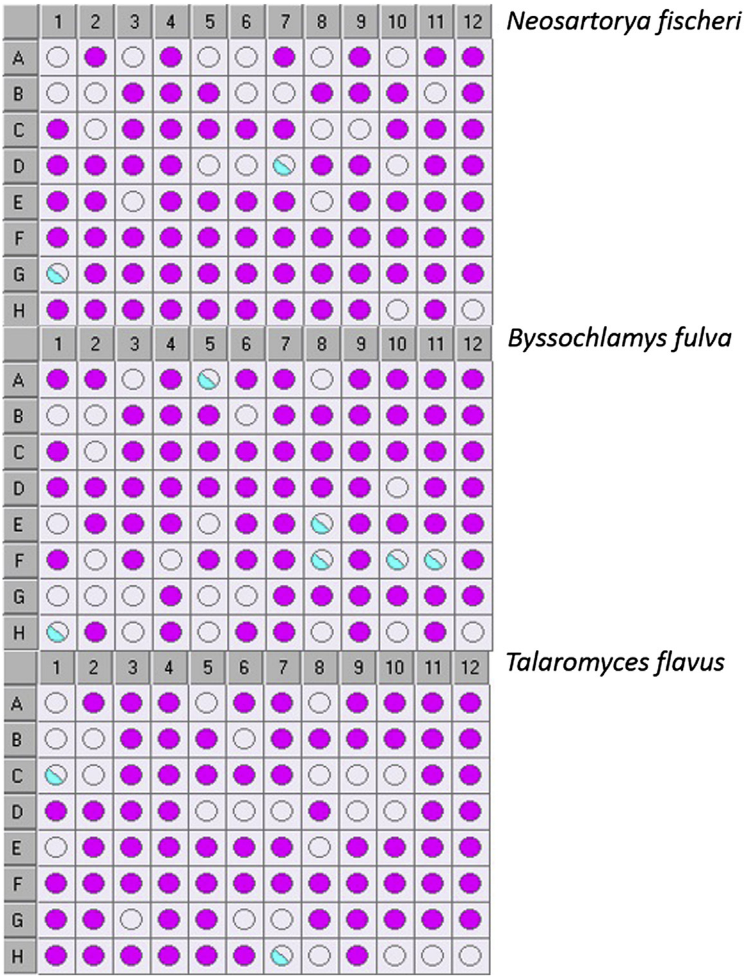

Figure 6 Carbon substrates utilized by heat-resistant fungi using FF Biolog Plates.

mycotoxins, especially patulin, a typical for *B. fulva* and verruculogen for *N. fischeri* were described.

Mycotoxins are important agents in relation to contamination of food and raw materials, including fruit. These products are not directly essential for growth, so they are called as secondary metabolites (Magan and Aldred, 2007). The study and knowledge about the mycotoxins is especially important due to the special threat of these compounds on the human food production chain (Frąc et al., 2010). According to Smedsgaard (1997), some of toxigenic metabolites can be considered as extrolites which are produced by different spoilage, including heat-resistant fungi and which can be helpful in

Table 2 Biolog FF Plate carbon source guild groupings (Frąc, 2012)

Well number	Carbon source	Compounds group
A1	Water	–
A2	Tween 80	Polymers
A3	N-Acetyl-D-galactosamine	Carbohydrates
A4	N-Acetyl-D-glucosamine	Carbohydrates
A5	N-Acetyl-D-mannosamine	Carbohydrates
A6	Adonitol	Carbohydrates
A7	Amygdalin	Miscellaneous
A8	D-Arabinose	Carbohydrates
A9	L-Arabinose	Carbohydrates
A10	D-Arabitol	Carbohydrates
A11	Arbutin	Carbohydrates
A12	D-Cellobiose	Carbohydrates
B1	α-Cyclodextrin	Polymers
B2	β-Cyclodextrin	Polymers
B3	Dextrin	Polymers
B4	i-Erythritol	Carbohydrates
B5	D-Fructose	Carbohydrates
B6	L-Fucose	Carbohydrates
B7	D-Galactose	Carbohydrates
B8	D-Galacturonic acid	Carboxylic and ketonic acids
B9	Gentiobiose	Carbohydrates
B10	D-Gluconic acid	Carboxylic and ketonic acids
B11	D-Glucosamine	Amines and amides
B12	α-D-Glucose	Carbohydrates
C1	Glucose-1-phosphate	Miscellaneous
C2	Glucuronamide	Amines and amides
C3	D-Glucuronic acid	Carboxylic and ketonic acids
C4	Glycerol	Miscellaneous
C5	Glycogen	Polymers
C6	m-Inositol	Carbohydrates
C7	2-Keto-D-gluconic acid	Carboxylic and ketonic acids
C8	α-D-Lactose	Carbohydrates
C9	Lactulose	Carbohydrates
C10	Maltitol	Carbohydrates
C11	Maltose	Carbohydrates
C12	Maltotriose	Carbohydrates
D1	D-Mannitol	Carbohydrates
D2	D-Mannose	Carbohydrates
D3	D-Melezitose	Carbohydrates
D4	D-Melibiose	Carbohydrates
D5	α-Methyl-D-galactoside	Carbohydrates
D6	β-Methyl-D-galactoside	Carbohydrates

(Continued)

Table 2 Biolog FF Plate carbon source guild groupings (Frąc, 2012)—cont'd

Well number	Carbon source	Compounds group
D7	α-Methyl-D-glucoside	Carbohydrates
D8	β-Methyl-D-glucoside	Carbohydrates
D9	Palatinose	Carbohydrates
D10	D-Psicose	Carbohydrates
D11	D-Raffinose	Carbohydrates
D12	L-Rhamnose	Carbohydrates
E1	D-Ribose	Carbohydrates
E2	Salicin	Miscellaneous
E3	Sedoheptulosan	Carbohydrates
E4	D-Sorbitol	Carbohydrates
E5	L-Sorbose	Carbohydrates
E6	Stachyose	Carbohydrates
E7	Sucrose	Carbohydrates
E8	D-Tagatose	Carbohydrates
E9	D-Trehalose	Carbohydrates
E10	Turanose	Carbohydrates
E11	Xylitol	Carbohydrates
E12	D-Xylose	Carbohydrates
F1	γ-Amino-butyric acid	Amino acids
F2	Bromosuccinic acid	Miscellaneous
F3	Fumaric acid	Carboxylic and ketonic acids
F4	β-Hydroxy-butyric acid	Carboxylic and ketonic acids
F5	γ-Hydroxy-butyric acid	Carboxylic and ketonic acids
F6	p-Hydroxyphenylacetic acid	Carboxylic and ketonic acids
F7	α-Keto-glutaric acid	Carboxylic and ketonic acids
F8	D-Lactic acid methyl ester	Miscellaneous
F9	L-Lactic acid	Carboxylic and ketonic acids
F10	D-Malic acid	Carboxylic and ketonic acids
F11	L-Malic acid	Carboxylic and ketonic acids
F12	Quinic acid	Carboxylic and ketonic acids
G1	D-Saccharic acid	Carboxylic and ketonic acids
G2	Sebacic acid	Carboxylic and ketonic acids
G3	Succinamic acid	Amines and amides
G4	Succinic acid	Carboxylic and ketonic acids
G5	Succinic acid mono-methyl ester	Miscellaneous
G6	N-Acetly-L-glutamic acid	Carboxylic and ketonic acids
G7	Alaninamide	Amines and amides
G8	L-Alanine	Amino acids
G9	L-Alanyl-glycine	Amino acids
G10	L-Asparagine	Amino acids
G11	L-Aspartic acid	Amino acids
G12	L-Glutamic acid	Amino acids

Table 2 Biolog FF Plate carbon source guild groupings (Frąc, 2012)—cont'd

Well number	Carbon source	Compounds group
H1	Glycyl-L-glutamic acid	Amino acids
H2	L-Ornithine	Amino acids
H3	L-Phenylalanine	Amino acids
H4	L-Proline	Amino acids
H5	L-Pyroglutamic acid	Amino acids
H6	L-Serine	Amino acids
H7	L-Threonine	Amino acids
H8	2-Amino ethanol	Amines and amides
H9	Putrescine	Amines and amides
H10	Adenosine	Miscellaneous
H11	Uridine	Miscellaneous
H12	Adenosine-5′-Monophosphate	Miscellaneous

identification and differentiation of related species by using microextraction methods. Heat-resistant fungi can produce a variety number of mycotoxins and other toxic metabolites such as patulin, byssochlamic acid, or fumitremorgins (Beuchat and Pitt, 2001). Because of the lack of monitoring, till now there is a little evidence that mycotoxins are produced in commercially processed products.

The fungal species are able to colonize specific ecological niches, including food products, fruits, raw agricultural materials, and soils due to their capability for effective competition against other microorganisms (Nielsen et al., 1988). Fungi can compete very effectively because of their ability to release a wide range of extracellular enzymes. This is an important factor of the competitiveness of fungal strain among others like temperature, water availability, pH, or gas balance. Stress connected with above-mentioned factors may result in changes of fungal community structure. In order to achieve such changes, fungi use different strategies to survive in difficult conditions. One of these strategies is secondary metabolites production. That is the way the possible role of secondary metabolites like mycotoxins must be considered in a wider ecological context (Magan and Aldred, 2007). There are many studies (Li, 2011; Salomao et al., 2007; Sant'Ana et al., 2010) on production of mycotoxins by spoilage, including heat-resistant fungi. The study presented by Puel et al. (2005) showed that mycophenolic acid kinetics by *B. nivea* was correlated with biomass with rapid early production (in the first 10 days) increasing over 30 days before decreasing, probably because of nutrient depletion. It is necessary to develop study on mycotoxins production in late exponential

and stationary phase due to the probability that fungi for colonizing food materials/products have to produce secondary metabolites earlier. If fungi colonize a food of matrix without competition due to the lack of different organisms, then they still often produce mycotoxins very rapidly, in spite of traditional point of view that they are able to mycotoxins production when there are no nutrients in the environment (Magan and Aldred, 2007).

Other important problem is food preservation, because some of filamentous fungi can be able to utilize components of preservatives added to the food products at the recommended level (Marin et al., 2002). This suggests that antioxidants and natural preservatives are able to stimulate the fungal growth so it is necessary to consider such impacts on shelf life of food products. There is only few studies concerning the metabolic profile and different substrates utilization by heat-resistant fungi and such study could be useful in the assessment of the risk of fungi development in foods and the selection of preservatives and agents that inhibit growth and development of these pathogens. Metabolomic production profiles for mycotoxins and other secondary metabolites could be useful tools for risk evaluation when there is a probability of food contamination by spoilage fungi, which compete from other microorganisms to be alive. Aldred et al. (2005) showed that ecological niches from which fungi are isolated significantly influence metabolic profiles and mycotoxins production. Thus the mycotoxins and different secondary metabolites production should be evaluated in ecological context, especially for heat-resistant fungi.

All fungi can produce extrolites, but fortunately only some of them are toxic. Some mycotoxins are acutely toxic, some are chronically toxic, and some are both. Different species produce different profiles of secondary metabolites (Frisvad and Samson, 2004). Figures 7 and 8 show the main mycotoxins produced by *N. fischeri* and *B. fulva*, respectively, the most important heat-resistant fungi in spoilage of juices and fruit products. Knowledge of the different species physiology is important not only regarding growth lag phase and growth rate, but also regarding conidia production and different metabolites utilization and production, including mycotoxins (Frisvad et al., 2007). Sometimes one toxin can be produced only on the specific substrate or medium and it cannot be produced in food products even after fungal contamination. Mycotoxins and other extrolites may be produced in large amounts on some media and not on the other, thus detailed knowledge of the influence of the substrate on mycotoxins production is crucial and needed.

Below we provide an overview of the most important mycotoxins which are produced by heat-resistant fungal species relevant in spoilage juices and fruit products.

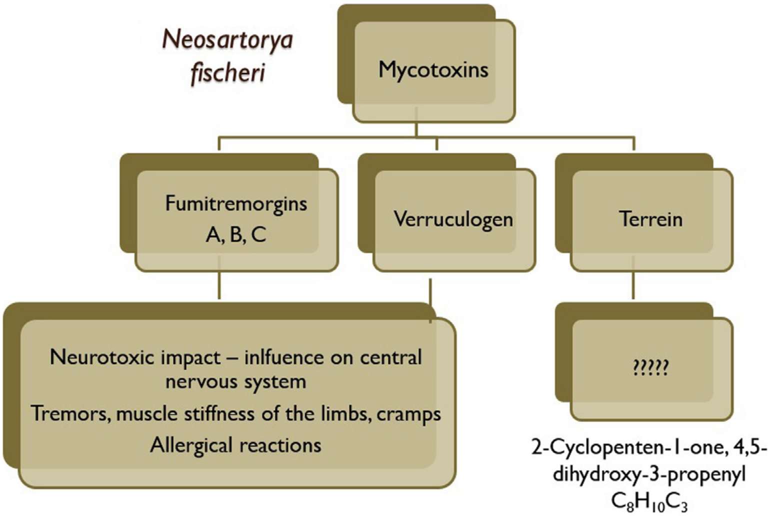

Figure 7 The main mycotoxins produced by *Neosartorya fischeri*.

6.1 The Main Mycotoxins Produced by *B. fulva* Species

6.1.1 Patulin

Patulin is a mycotoxin produced by species belonging to genera of *Penicillium*, *Aspergillus*, and heat-resistant *Byssochlamys* (Moake et al., 2005; Sant'Ana et al., 2008, 2010). For the first time, patulin was isolated from *Penicillium griseofulvum* and *Penicillium expansum* in 1943 (Birkinshaw et al., 1943).

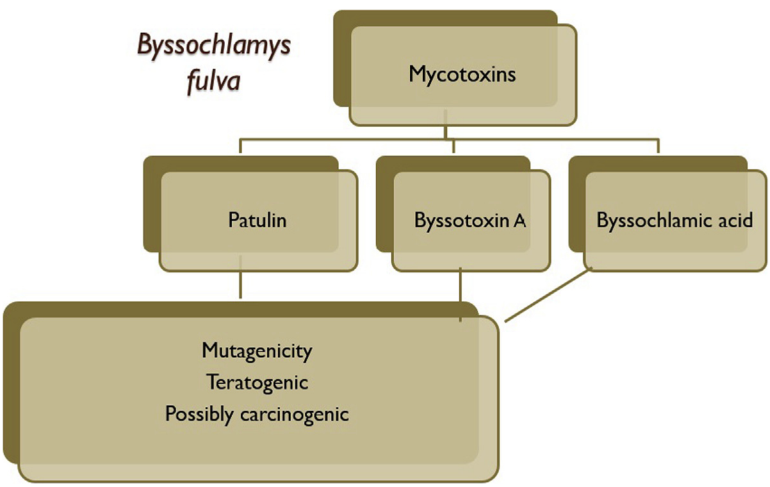

Figure 8 The main mycotoxins produced by *Byssochlamys fulva*.

Taking under consideration the chemistry of patulin it is a polyketide lactone that is heat-stable and not destroyed by thermal denaturation and pasteurization (Puel et al., 2010).

In general, patulin is very toxic because of prokaryotes as well as eukaryotes. Toxicity of patulin is connected with the affinity of this toxin for sulphydryl groups. In general, patulin toxicity concerns the acute and subacute toxicity, genotoxicity, embryotoxicity, and teratogenicity (Puel et al., 2010). Symptoms of acute toxicity caused by patulin include convulsions, dyspnea, pulmonary congestion, edema, and ulceration gastrointestinal tract, whereas symptoms of subacute toxicity include tremors, convulsions, changes in the hormones level, and histological dysfunctions (Selmanoglu, 2006). Several studies confirmed genotoxicity of patulin (Liu et al., 2003) by structural chromosomal aberrations, genes mutations and chromosome gaps and breaks. It is necessary to note that most of the studies on genotoxicity on mammalian cells were positive, while on bacteria it was mainly negative (Puel et al., 2010). Patulin is known as embryos defective agent by the proteins and DNA content reduction. Anomalies include growth retardation, hypoplasia and hyperplasia of embryos (Smith et al., 1993). Whereas according to International Agency for Research on Cancer, patulin is not classified as carcinogenicity agent for human.

Patulin belongs to mycotoxins, of which level in food is regulated in many European countries and also in the United States. The maximum level of patulin in food products and juices allowed by European Union is 50 $\mu g\ kg^{-1}$ (CodexAlimentarius, 2003) and 10 $\mu g\ kg^{-1}$ for babies and young infants products (EuropeanUnion, 2006). The United States limits patulin to 50 $\mu g\ kg^{-1}$. The highest risk of contamination by patulin is connected with apple industry producing juices, cider, compotes, jams, mousses, food intended for young children, and other apple-derived products (Puel et al., 2010). Patulin was also found in other foods like grains, cereals, fruits, and vegetables (Hopmans, 1997; Rodriguez-Carrasco et al., 2014). Different species of *Penicillium* are known as producers of patulin in pure cultures, cereals, pasta, beer, wine, and meat products (Frisvad et al., 2007). Patulin production by *P. expansum* was widely studied in culture media (Dombrink-Kurtzman and Blackburn, 2005; Kokkonen et al., 2005) and fruit juice (Morales et al., 2008; Tournas and Memon, 2009).

There are also some studies concerning the patulin production by heat-resistant mold species belonging to the *Byssochlamys* genus (Houbraken et al., 2006; Puel et al., 2007; Rice, 1980; Rice et al., 1977; Sant'Ana et al., 2010). The study on patulin production by heat-resistant fungi included two

species *B. fulva* and *B. nivea*. The results concerning *B. fulva* are not unequivocal. The first study based on data with fruit juices from the 1970s to 1980s indicated that both *B. nivea* and *B. fulva* species were able to produce patulin (Rice, 1980; Rice et al., 1977). Data presented by Houbraken et al. (2006) and Puel et al. (2007) were in contrary to previous analyses and indicated that *B. fulva* was not able to produce this mycotoxin in culture media. The most recent study reported by Sant'Ana et al. (2010) confirmed by using three different wavelengths by high-performance liquid chromatography and UV detection that both *B. nivea* and *B. fulva* strains produced patulin after fruit juice inoculation. Moreover, the ability of patulin production by *Byssochlamys* is connected with the presence of the idh gene (Dombrink-Kurtzman and Engberg, 2006; Puel et al., 2007), and this gene was also detected in *B. fulva* strains isolated from soil (Paterson, 2004). This is important and suggests that patulin production has to be taken at risk not only for *B. nivea* but also for *B. fulva* strains. It is known that many factors like carbon sources, pH, juice type, temperature, water activity, gas balance, and presence of preservatives may strongly affect filamentous fungi growth, metabolism, and patulin production (Kokkonen et al., 2005; Sant'Ana et al., 2010). The study of Sant'Ana et al. (2010) indicated that patulin production occurred within 10 days after juice pasteurization causing a risk to consumers. Thus it is necessary to consolidate the control monitoring to reduce contamination of raw materials and juices by ascospores of heat-resistant fungi such as *Byssochlamys*.

6.1.2 Byssotoxin A

Byssochlamys fulva, isolated from corn, was able to produce a very specific toxin—byssotoxin A, which was characterized chemically and physically by Kramer et al. (1976). The toxicity of byssotoxin A was found for brine shrimp, chicken embryos, and rats. Crude extracts were more toxic than pure toxin (Kramer et al., 1976). Compared to byssochlamic acid, which characterized high toxicity (Houbraken et al., 2006), byssotoxin A is known as toxin with the intermediate toxicity level. Kramer et al. (1976) indicated its slightly inhibiting influence on pea seeds germination, but they did not observe any toxicity to bacteria and yeast strains.

Taking under consideration the chemical properties of byssotoxin A, Kramer et al. (1976) indicated that this toxin was insoluble in acetone, benzene, and hexane, slightly soluble in chloroform, ethanol, and water and soluble in methanol and 5% NaOH. Byssotoxin A was found as a single orange to orange-red on thin-layer chromatography plates. Nitrogen analysis

showed that the compound contained 2 mol of nitrogen per mol of compound. The structure of byssotoxin A was not determined. Fragmentation analysis indicated that byssotoxin A consists of a large stable ring structure with a chain of eight or nine carbons that terminates in an amine or nitrosamine group (Kramer et al., 1976). As it was written above, Kramer et al. (1976) reported that *B. fulva* produced byssotoxin A, but because of the structure of this mycotoxin, study of presence and production by heat-resistant mold were not continued later.

6.1.3 Byssochlamic Acid

Byssochlamys fulva is able to produce byssochlamic acid (Kramer et al., 1976; Rice et al., 1977). Byssochlamic acid was also recognized as a mycotoxin produced by *B. nivea*, *B. lagunculariae*, and *B. verrucosa* (Houbraken et al., 2006) among *Byssochlamys* species. Byssochlamic acid belongs to the group of nonadrides characterized by the presence of anhydride groups attached to a nine-membered carbocyclic ring ($C_{18}H_{24}O_8$). It is known that byssochlamic acid is characterized by high toxicity and hemorrhagicity. Raistrick and Smith (1933) showed that byssochlamic acid was toxic to mice. It was also confirmed its hepatotoxicity to guinea pig (Beuchat and Rice, 1979). The study presented by Schmidt and Rehm (1970) indicated that byssochlamic acid inhibited the primary metabolism of several enzymes like adenosine deaminase, alcohol dehydrogenase, and isocitrate hydrogenase. Byssochlamic acid salt had negative influence on *Sinapis alba* germination and *Saccharomyces cerevisiae* fermentation (Meyer and Rehm, 1969). The cytotoxicity of the natural byssochlamic acid is not known. However, its enantio isomer has moderate cytotoxic activity against HEp-2 and HepG2 cells in vitro (Li et al., 2007). It could indicate a possible toxic effect of the natural byssochlamic acid. Production of this toxin by *Byssochlamys* species was confirmed in microbiological media but also in food and feed (Houbraken et al., 2006).

Byssochlamic acid was produced on glucose, but toxin production was also increased on such substrates as sucrose, maltose, fructose, galactose, lactose, xylose, citric and gluconic acid, and glycerol (Beuchat and Rice, 1979). The study presented by King et al. (1972) showed that grape and tomato juice contaminated by *B. fulva* was free of byssochlamic acid after 2 months and 1 year, respectively. There are also few reports (Fritz et al., 1976) concerning the spoilage and food contamination by byssochlamic acid. Byssochlamic acid contamination was investigated not only in fruits and juices, but also in fats and fat-containing food. The production of this toxin was not observed in the presence of palmitic, stearic, and linoleic acids,

only oleic acid stimulated byssochlamic acid production. Taking under consideration fats such as margarine, olive oil, bacon fat, and palm oil, byssochlamic acid was not found after growth of *B. fulva* during 7 months, whereas this toxin was detected in butter (Schmidt and Rehm, 1969).

6.2 The Main Mycotoxins Produced by *N. fischeri* Species

6.2.1 Fumitremorgins

Fumitremorgins belong to the indole alkaloids group. These compounds were isolated from various fungal sources. These toxigenic metabolites are produced by heat-resistant fungi, especially by *N. fischeri* strains, which produce three types of these toxins: fumitremorgin A, B, and C (Frisvad and Samson, 1991; Hong et al., 2005; Nielsen et al., 1988). Fumitremorgins production by *N. fischeri* is not directly depended on conidia formation. These toxins can be produced by *N. fischeri* in heat-processed fruit products under a wide range of environment conditions like pH, temperature, light, and oxygen (Nielsen et al., 1988). Frisvad and Samson (1991) showed that minimal water activity to fumitremorgins production by *N. fischeri* is 0.955 for fumitremorgin A and 0.925 for fumitremorgin C. Tremogenic fumitremorgins can be produced even at low oxygen concentration (0.1%) and increase of its production is connected with the presence of carboxylic acids like citric, malic, or tartaric in a medium (Nielsen et al., 1988). Besides heat-resistant molds fumitremorgins are also produced by the following species: *Aspergillus caespilosus*, *Penicillium mononematosum*, and *Plasmodium brasilianum*, but these fungi are very rare in food and feeds (Frisvad et al., 2006) and *A. fumigatus* (Hong et al., 2005). Fumitremorgins act on the central nervous system causing tremors, convulsions, and death of animals (Patterson et al., 1981; Yamazaki et al., 1979, 1971). The study of Yamazaki and Suzuki (1986) and Yamazaki et al. (1983) indicated that fumitremorgins A and B were acute toxic and neurotoxic for mice. Lethal dose of this toxin was at the following level: $LD_{50} > 25\ mg\ kg^{-1}$ body weight. From the one hand, they are toxic for human health, but because of their intriguing chemical structure they could have interesting biological and pharmaceutical activities (Li, 2011).

6.2.2 Verruculogen

Verruculogen similar as fumitremorgins is a tremorgenic toxic with the same indole activity. Verruculogen is extremely toxic, but not very common in food. It is known that this toxin is produced by strains belong to the *Penicillium* genus (Pitt and Hocking, 1997), especially by *P. brasilianum* (Frisvad and Samson, 2004), but there are no reports of its occurrence in natural

environment, for example, in food and feeds. About above-mentioned fungal species, verruculogen is also produced by *Aspergillus caespitoyus*, *Pythium graminicola*, and *P. mononematosum* (Frisvad et al., 2007). The verruculogen production by heat-resistant *N. fischeri* strains was confirmed by Nielsen et al. (1988) and Frisvad and Samson (1991). Other authors (Khoufache et al., 2007) indicated that verruculogen is formed also by *A. fumigatus*. The study presented by Nielsen et al. (1988) indicated that verruculogen was produced by *N. fischeri* strains at high level on various microbiological media. However, till now there is only fragmentary information about the production and significance of this toxin in food. Verruculogen can act on nervous system causing tremors in swine and sheep. Even small doses of this toxin are dangerous (5−10 μg kg^{-1} body weight) what was reported by Perera et al. (1982).

6.2.3 Terrein

Terrein is a bioactive fungal metabolite whose effects are almost unknown, but its structure is the following: 2-cyclopenten 1-one 4,5-polihydroxy 3-propenyl ($C_8H_{10}C_3$). There is no study on the toxic effect of this toxin to human health. The study of Park et al. (2004) indicated that terrein is known to potently inhibit melanin production in melanocytes. Kim et al. (2007) showed that terrain has a strong antiproliferative influence on human skin equivalents, what suggests that could be developed as a treatment for hyperproliferative skin diseases. Terrein is produced by *Aspergillus novofumigatus* and *Aspergillus lentulus*, closely related to heat-resistant *N. fischeri* species, as one from many extrolites like hevolic acid, neosartorin, and palitantin. However, this toxin was not found during growing the culture of *A. fumigatus*, *Aspergillus fumigatiaffinis* and *N. fischeri* (Hong et al., 2005). Geiser et al. (1998) indicated that phylogenetic relationships based on DNA sequence are not in agreement with phylogenesis suggested by extrolites. The study of Hong et al. (2005) showed that heat-resistant *N. fischeri* was not able to produce terrein, but many extrolites like aszonalenin, fiscalins, fischerin, helvolic acid, neosartorin, pyripyropens, trypacidin, tryptoquivalins, and tryptoquivalons were formed by this species.

The study carried out over last few decades indicated that mycotoxins play a significant role in the food safety. Because agriculture is a dominant component of global economy, increase in the production of heat-processed products and safe high-quality food is relevant for the world's population. Moreover, metabolites of heat-resistant fungal species (mycotoxins) will continue to be critical interest of researchers for next few years.

7. THE CHARACTERIZATION OF INACTIVATION KINETICS (D-VALUES) AND CONTROL METHODS OF HEAT-RESISTANT FUNGI

D-value is the most commonly used mathematical measurement of death rate. D-value is defined as the time required at a given temperature to reduce one log cycle of the microorganisms. Z-value defined as the degree of temperature required to bring about a 10-fold change in the death rate, is used to convert a D-value for one temperature to that of another (Salomao et al., 2007). Ascospores of heat-resistant fungi like *Neosartorya*, *Byssochlamys*, and *Talaromyces* can survive temperatures that range between 55°C and 95 °C. Scholte et al. (2004) reported a decimal reduction time of 1.5−11 min at 90 °C for some of them. Many heat-resistant fungi isolated from different environments have different D-values in various conditions. Beuchat (1986) evaluated the resistance to the heat used in the processing of various products and temperatures of heat-resistant ascospores of *T. flavus* and *N. fischeri* on fruit-based products like blueberry, cherry, peach, raspberry, and strawberry. Ascospores of *N. fischer*i were less heat resistant than *T. flavus*. Heat resistance varies between fruit but the author did not find any correlation between measured parameter (pH, soluble solid s) and degree of heat resistance. In contrary, the study presented by Quintavalla and Spotti (1993) indicated that *N. fischeri* ascospores were more heat resistant than those of the other species, *Talaromyces* and *Byssochlamys*. Ascospores of *B. nivea* were considerably less heat resistant.

The strains of *Talaromyces macrosporus*, *N. fischeri*, and *B. nivea* indicate that these organisms may survive commercial heat processes. Heat resistance of heat-resistant fungi at different temperatures and media were presented in Table 3. Germination of heat-resistant ascospores is activated and synchronized by a heat treatment (Dijksterhuis and Samson, 2002). The ascospores of heat-resistant fungi belong to the *Talaromyces* genus, contain high level of trehalose, low water amount, and are bound by a very thick multilayered cell wall. Ascospores of *Neosartorya* species seem to germinate by a slow separation of the two shell-like ornamented halves and subsequent formation of a germ tube (Dijksterhuis and Samson, 2002). The study of Reyns et al. (2003) indicated that the structural changes occurring in the cell wall have direct impact on the ascospores activation process.

Thermal conditions required to inactivate ascospores of heat-resistant fungi in fruit products in general decrease the sensory quality (Butz et al., 1996). Food processing using high pressures is a nonthermal method

Table 3 Heat resistance of heat-resistant fungi at different temperatures and media

Fungal species	Temperature (°C), D-value (min), z-value (°C)	Medium	References
Neosartorya fischeri	85, 17.6, 6.1 85, 47, 5 85, 116, 3	Blueberry	Beuchat (1986)
	85, 19.2, 5.3 85, 41.2, 4.1 85, 51.8, 3.3	Cherry	Beuchat (1986)
	85, 15.1, 6 85, 36.9, 3.9 85, 43.9, 3.8	Peach	Beuchat (1986)
	85, 16.4, 5.3 85, 30.1, 5.1 85, 41.1, 3.9	Raspberry	Beuchat (1986)
	85, 19.4, 5 85, 41.4, 4.9 85, 45, 3.2	Strawberry	Beuchat (1986)
	85, 5.1, – 85, 5, 4.7 85, 29.4, 5.1 85, 35.8, 3.5	Grape juice	Quintavalla and Spotti (1993)
	80, 208.3, 5 85, 30.1, 5 90, 2.0, 5	Apple juice	Salomao et al. (2007)
	80, 129.9, 5.5 85, 19.0, 5.5 90, 1.9, 5.5	Papaya juice	Salomao et al. (2007)
	80, 73.5, 5.9 85, 13.2, 5.9 90, 1.5, 5.9	Pineapple juice	Salomao et al. (2007)
Talaromyces flavus	85, 64.2, 5.9 85, 9.7, 9.2 85, 68.1, 5.4	Blueberry	Beuchat (1986)
	85, 20.4, 9.6 73, 9.2, 12.9 85, 26.5, 7.7	Cherry	Beuchat (1986)
	85, 26.9, 7 73, 11.3, 7.8 85, 28.5, 6.9	Peach	Beuchat (1986)
	85, 28.8, 6.5 73, 8.9, 9.4 85, 26.2, 5.7	Raspberry	Beuchat (1986)

Table 3 Heat resistance of heat-resistant fungi at different temperatures and media—cont'd

Fungal species	Temperature (°C), D-value (min), z-value (°C)	Medium	References
	85, 47.1, 5.2 73, 9.4, 9.2 85, 52, 5.3	Strawberry	Beuchat (1986)
Byssochlamys nivea	80, 4.2, – 80, 6.6, 5.8 80, 10, 4.2 80, 15.7, 4.5 80, 32, 3.9	Grape juice	Quintavalla and Spotti (1993)
	80, 15.4, – 85, 2.0, –	Apple juice	Hosoya et al. (2012)
	80, 13.8, – 85, 1.4, –	Saline solution	Hosoya et al. (2012)
Talaromyces macrosporus	85, 7, 4 85, 2.1, 4.7 85, 34.7, 6.6 85, 29.6, 4.8	Grape juice	Quintavalla and Spotti (1993)
Byssochlamys fulva	80, 89.5, – 85, 16.7, –	Apple juice	Hosoya et al. (2012)
	80, 70.6, – 85, 12.3, –	Saline solution	Hosoya et al. (2012)
Byssochlamys lagunculariae	80, 5.0, – 85, 0.4, –	Apple juice	Hosoya et al. (2012)
	80, 4.5, – 85, 0.4, –	Saline solution	Hosoya et al. (2012)
Thermoascus aegyptiacus	90, 56.2, –	Glucose–tartrate solution	Hosoya et al. (2014)
Thermoascus aurantiacus	80, 57.1, 5.2 83, 13.2, 5.2 85, 10.8, 5.2	Glucose–tartrate solution	Hosoya et al. (2014)
Thermoascus thermophilus	90, 21.3, –	Glucose–tartrate solution	Hosoya et al. (2014)

for the high-quality production. Additionally, vitamins are better preserved after the application of this alternative preservation method (Hocking et al., 2006). High pressure processing (HPP) is useful especially for acidic food like fruit products and juices. HPP controls and inactivates microorganisms through changes to their biochemistry, morphology, and gene function. There are many factors influencing the HPP effectiveness.

Among others, the most important are application time, organism's species or strains, growth stage and age of the fungal culture, and the medium composition (San Martin et al., 2002). In general, vegetative cells of fungi are regarded as sensitive to HPP, however, ascospores of heat-resistant fungi are considered pressure resistant (Chapman et al., 2007). Heat-resistant fungi are the most dangerous for processed fruit, especially after thermal processing (Voldrich et al., 2004). The study presented by Hocking et al. (2006) indicated that blanching at 95 °C for 5 min did not affect the pressure resistance and increased the resistance to pressure. HPP (600 MPa for several minutes) was sufficient to inactivate vegetative cells of *N. fischeri* and *B. fulva* strains. However, it was insufficient to inactivate even relatively young ascospores of heat-resistant fungi. The combined methods (thermal and high pressure) could be effective in controlling the outgrowth of these fungi during fruit processing. Palou et al. (1998) showed no inactivation of *B. nivea* ascospores after pressure cycles (1, 3, or 5 cycles at >600 MPa, 1 s time, at 21 °C). However, Ferreira et al. (2009) indicated that for inactivation of ascospores of heat-resistant *B. nivea* in pineapple juice and nectar, sequence pressure cycles was more effective than the use of sustained high pressures. The three (5 min) or five (3 min) pressure cycles (600 MPa, 80 °C) could inactivate 10^5-10^6 CFU mL^{-1} of *B. nivea* ascospores, so it could be sufficient for pasteurization in some case of manufacturing conditions. Moreover, taking under consideration organoleptic properties of fruit products and passion fruit juices (Marcellini et al., 2006), pressure preserves its overall sensory quality compared to pasteurization (Laboissiere et al., 2007). Form the practical point of view, the most important observation reported by Dijksterhuis and Teunissen (2004) was the activation of ascospores germination by even a very short treatment at high pressure. It is important to combine the elevated temperature application together with the presence of high pressures because it can effectively kill the ascospores. Because minimal thermal processing is desirable for nutritional and natural organoleptic products quality, preservatives and acidulants are used to protect juices against heat activation of ascospores. Acidulants and chemical preservatives such as lactic, malic, citric, and tartaric acids and sodium benzoate, potassium sorbate can increase the shelf life of thermally processed fruit products (Rajashekhara et al., 2000). However, to control heat-resistant molds in fruit-processing plants, it is necessary to use high-quality fruits which have been adequately monitored, washed, disinfected, and maintaining hygienic conditions.

8. FUTURE RESEARCH NEEDS

There will always be a need for an understanding of the occurrence of heat-resistant fungi in raw materials like fruits and vegetables and food products and the effectiveness of treatment processes for their protection. New heat-resistant fungi will continue to emerge and methods for their detection in the environment will be developed. The most important one will be based on molecular biology methods, which are very sensitive and precise. The nondestructive methods in these purposes will also be developed. Newer and better treatment processes of heat-resistant fungi inactivation will be developed to increase the quality of food products. Identification of heat-resistant fungi by conventional methods is long lasting and based on the ascospores formation and differentiation of their microstructure. Because these methods are time consuming, it is necessary to develop novel, rapid, and convenient identification and detection methods.

Most of our understandings about heat-resistant fungi are connected with resistance to different temperatures, acidulants, and preservatives, but there are only a few studies on the mechanisms of heat resistance of these fungi. Modern techniques based on microscopy and spectroscopy will be developed in order to explain the heat resistance of these pathogens. Since many of heat-resistant fungi produce mycotoxins and other biological active extrolites, we need to assess the toxicity and positive biological effects of these metabolites in the environment. Potential of utilizing different sources of substrates and chemical sensitivity can be useful in the elaboration of fungicides, which will be dedicated to heat-resistant fungi inactivation. It could decrease the risk of raw materials contamination by heat-resistant fungi from soil. PMs and phylogenetic analyses will be developed to assess the presence of new species of these pathogens and to evaluate the relationships and metabolic abilities of heat-resistant fungi. Combination of molecular analysis of the heat-resistant fungi and functional analysis of their biochemical processes allows understanding the mechanisms of heat resistance and heat-resistant ascospores formation. Identification of a gene encoding heat resistance and the evaluation of their expression in different species and conditions will be very interesting for the future.

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